Nanotechnology in the Life Sciences

Vibhav Gautam
Rajiv Kumar
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Nanomedicine

Innovations, Applications, and Breakthroughs in the Quest for Health and Medicine's Future

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Nanotechnology in the Life Sciences

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Nano and biotechnology are two of the 21st century's most promising technologies. Nanotechnology is demarcated as the design, development, and application of materials and devices whose least functional make up is on a nanometer scale (1 to 100 nm). Meanwhile, biotechnology deals with metabolic and other physiological developments of biological subjects including microorganisms. These microbial processes have opened up new opportunities to explore novel applications, for example, the biosynthesis of metal nanomaterials, with the implication that these two technologies (i.e., thus nanobiotechnology) can play a vital role in developing and executing many valuable tools in the study of life. Nanotechnology is very diverse, ranging from extensions of conventional device physics to completely new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale, to investigating whether we can directly control matters on/in the atomic scale level. This idea entails its application to diverse fields of science such as plant biology, organic chemistry, agriculture, the food industry, and more.

Nanobiotechnology offers a wide range of uses in medicine, agriculture, and the environment. Many diseases that do not have cures today may be cured by nanotechnology in the future. Use of nanotechnology in medical therapeutics needs adequate evaluation of its risk and safety factors. Scientists who are against the use of nanotechnology also agree that advancement in nanotechnology should continue because this field promises great benefits, but testing should be carried out to ensure its safety in people. It is possible that nanomedicine in the future will play a crucial role in the treatment of human and plant diseases, and also in the enhancement of normal human physiology and plant systems, respectively. If everything proceeds as expected, nanobiotechnology will, one day, become an inevitable part of our everyday life and will help save many lives.

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Editors

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Foreword

With great joy and anticipation, I am delighted to present this extraordinary addition to Springer Nature's esteemed collection. This book opens the door to a vast realm of knowledge and exploration in the field of nanomedicine, showcasing innovations, applications, and breakthroughs that are shaping the future of health and medicine. It invites readers to grasp the crucial role and significance of nanomedicine across various industries.

Featuring contributions from distinguished scholars, this volume delves into numerous topics with potential applications in agriculture and pharmaceuticals. The chapters are meticulously arranged to provide a comprehensive understanding of nanomedicine's roles and importance in these fields.

This book serves as an invaluable resource for researchers and students studying nanomedicine, while also offering valuable reference material for professionals in the agriculture and pharmaceutical sectors. I extend my heartfelt appreciation to the editors and contributors who have poured their passion and expertise into creating this remarkable volume. Their collective contributions enrich the academic discourse that shapes our world.

Finally, I hope this book will inspire and empower readers to achieve new heights of expertise in nanomedicine and kindle a curiosity that drives remarkable advancements in this field.

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Shyam Sundar, MD, FNASc, FASc, FNA, FTWAS

Preface

Every book starts somewhere, and this one is no exception. As we explore the compiled chapters on various aspects of nanomedicine, we are reminded of its crucial significance in agriculture, health, and pharmaceutical industries. This preface outlines the reasons behind creating this book on "*Nanomedicine—Innovations, Applications, and Breakthroughs in the Quest for Health and Medicine's Future*."

This book emerged from numerous experiences, discussions, and a deep-seated desire to share insights gained about nanomedicine. It features contributions from leading scholars exploring the potential of nanomedicine.

Within these pages, you will discover chapters exploring different facets of nanomedicine that may challenge, provoke thought, or inspire deeper exploration of this dynamic field. Certain chapters may encourage reflection and prompt you to contribute your own ideas to the discussion.

Ultimately, this book aims to share ideas, perspectives, and the latest advancements in nanomedicine. We hope it serves both as a valuable resource for learning and as a tool for further research in this rapidly evolving area of study.

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Acknowledgments

The completion of this volume, "*Nanomedicine—Innovations, Applications, and Breakthroughs in the Quest for Health and Medicine's Future*," marks a significant milestone, and we sincerely appreciate the individuals whose contributions have made this achievement possible.

First and foremost, we extend heartfelt appreciation to all the contributors who participated in this book. Your expertise and invaluable insights have played a crucial role in shaping the content and scope of this volume. We also express gratitude to the Springer Nature team for their continuous support and collaboration in finalizing this work.

Lastly, we thank the readers who will join us on this journey. We hope the contents of this volume meet your expectations and provide valuable insights into the field.

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Nanomedicine Approaches in Oral Cancer: From Diagnosis to Therapy

Akhilesh Kumar Singh, Mehul Shashikant Hirani, Vyomika Bansal, and Naresh Kumar Sharma

1 Introduction

“Nanotechnology” is a derived from Greek ‘nano’ which means ‘dwarf’ which was envisioned by the Nobel Prize winner physicist Richard Feynman in 1959 [1]. It is the manipulation of matter on the molecular and atomic levels that allows researchers with newer investigatory tools in the range of typically one billionth of a meter (i.e. 10^9), equal to 1 nm. This is the normative size for molecules that are biologically important, such as hemoglobin, proteins, receptors, enzymes and antibodies. Nanotechnology involves inherently small, synthetic nanoscale materials (1–100 nm range) utilized in medicine, allowing them to pass through cell membranes and blood vessel walls as well as biological barriers like the blood-brain barrier [2–5]. These nanoparticles (NP) exhibit tunable physical (mechanical, electrical, chemical, magnetic & optical) and biological properties that differ significantly from the larger size forms of similar materials [6]. Nano objects can have a variety of micro-shapes, such as spheres, tubes, rods, and wires, and can be impregnated with materials that are hollow, porous, or solid, such as lipid- or polymer-based, gold, or iron oxides, depending on the varied applications. Due to their strong interaction & transport capabilities, NPs are considered as suitable candidate for their application in the development of biological sensors, imaging contrast agents, and/or medicinal carriers [7–10]. Nanotechnology tools have a wide range of applications, from device physics to biology. The United States has been making a concentrated effort since the 1990s to establish such research initiative. Since then, enormous resources have been allocated to the advancement of cutting-edge nanotechnologies in an effort to lower the death rate from cancer [11]. Recently, the fields of medicine and nanotechnology

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Table 1 Generations of Nanotechnology

Generation	Constituents
I: Passive nano-structures	Dispersed & contact nanostructures Aerosols Colloids Products incorporating nanostructures Coatings Polymers Ceramics Nanostructure metals Nanoparticle reinforced composites
II: Active nano-structures	Bioactive, health effects-targeted drugs Bio-devices Physicochemical active structures Amplifiers Actuators Adaptive structures 3D transistors
III: Nano-systems	Guided assembling of structures 3D networking of structures New hierarchical architectures Robotics Evolutionary systems
IV: Molecular nano-systems	Molecular devices by design Molecular devices by atomic designing Molecular devices with emerging functions

have come together to form the multidisciplinary discipline of nanomedicine, which links doctors, engineers, physicists, biologists, chemists and physicists in the pursuit of better medication delivery, detection, and imaging technologies [12]. Among many other exciting developments, the application of nanotechnology has transformed biomedical engineering by enabling new drug delivery methods, the synthesis of tissue modules, the invention of biomaterial & improved medical device surfaces, in vitro & in vivo methods, biofiltration systems, and robotic assembly [13].

Materials with components less than 100 nm in a minimum of one dimension are referred to as nanomaterials. These combinations consist of atom clusters, grains, fibers, nanoholes & composites. Ravisankar et al. [14] has described various generations & constituents of nanotechnology as shown in Table 1.

This chapter discusses current advancements in the field of nanomedicine's significance in the diagnosis and treatment of oral cancer, which are made possible by these micro- & nanotechnologies.

2 Oral Cancer Diagnosis & Identification

Cancer of oropharyngeal region is very common and quite aggressive with local tissue invasion, regional metastasis and a high mortality rate [15]. Traditional method of cancer diagnosis is usually based on the identification of some involved proteins in the pathogenesis or the histological examination of tumor mass of a few cubic centimetres, under magnification which has about 10^8–10^9 of cancer cells [16]. Head and neck squamous cell carcinoma (HNSCC) with its subtype Oral Squamous Cell Carcinoma (OSCC) is 6th most commonly diagnosed cancer, although there is very little improvement in survival rate following medico-surgical management despite many advancements in medical field [17]. Frequently, oral cancer becomes apparent only after it has progressed to an incurable level, when cancer cells become destructive & unresponsive to treatment [18]. Patients inevitably have significant side effects of the treatment, therefore creating safer and more effective techniques is essential. Therefore, increasing the disease's survival rate requires early OSCC identification. Extracellular vesicles (EVs) and biometric nanoparticles (NPs) are two examples of nanomedicines that are currently used to treat patients effectively.

Knowing the mechanisms underlying the onset and spread of cancer may help us choose the best treatment plan at the appropriate time. Following an oral carcinogenic insult, there are multiple processes that lead to diverse chemical alterations that disrupt via cellular development, proliferation, and differentiation culminating into characteristic malignant cellular change & carcinogenesis (Fig. 1a). Similar to other cancers, oral carcinogenesis begins with an early premalignant lesion &/or condition and then gradually progresses to local-regional metastatic disease [19]. The extent of the disease spread from normal epithelium to hyperplasia/dysplasia is indicated by the histopathological grading of dysplasia. Changes in nuclear size & shape, enlarged cells and nuclei, enhanced mitotic appearance and increased nucleus/cytoplasm ratio are some of the frequently observed cellular events in

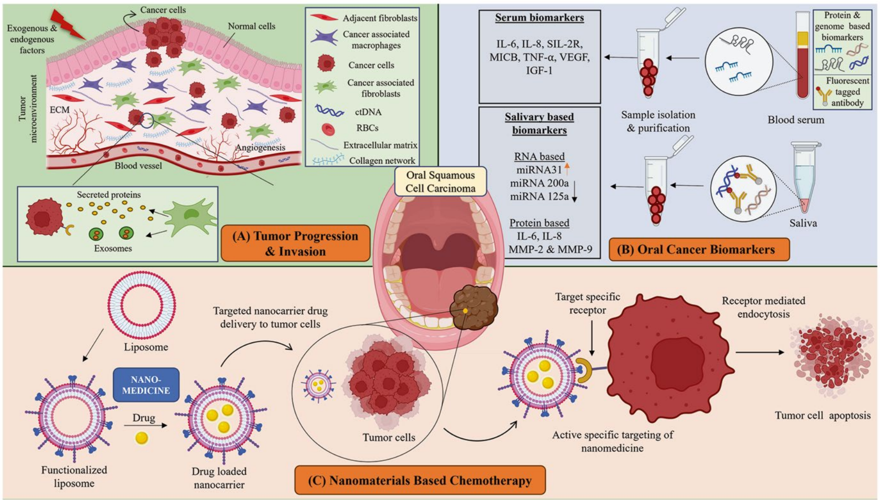
Exogenous & endogenous factors
Cancer cells
Normal cells
Tumor microenvironment
ECM
Angiogenesis
Blood vessel
Adjacent fibroblasts
Cancer associated macrophages
Cancer cells
Cancer associated fibroblasts
ctDNA
RBCs
Extracellular matrix
Collagen network
Secreted proteins
Exosomes
(A) Tumor Progression & Invasion
Oral Squamous Cell Carcinoma
Serum biomarkers
IL-6, IL-8, SIL-2R, MICB, TNF-α, VEGF, IGF-1
Salivary based biomarkers
RNA based
miRNA31 ↑
miRNA 200a ↓
miRNA 125a ↓
Protein based
IL-6, IL-8
MMP-2 & MMP-9
Sample isolation & purification
Blood serum
Protein & genome based biomarkers
Fluorescent tagged antibody
Saliva
(B) Oral Cancer Biomarkers
Liposome
NANO-MEDICINE
Drug
Functionalized liposome
Drug loaded nanocarrier
Targeted nanocarrier drug delivery to tumor cells
Tumor cells
Target specific receptor
Receptor mediated endocytosis
Active specific targeting of nanomedicine
Tumor cell apoptosis
(C) Nanomaterials Based Chemotherapy

Fig. 1 Diagrammatic illustration of Nano diagnostic approach in Oral Cancer. (**a**) Molecular basis for oral cancer progression and invasion. (**b**) Several biomarkers involved in early diagnosis of oral squamous cell carcinoma. (**c**) Nanomaterials based target specific drug delivery for oral cancer diagnosis

OSCC. Pathological tissue characteristics in later stages may show increased cellular density, basal cells hyperplasia, dyskeratosis, and bulbous rete pegs with secondary nodules. The manipulation, separation, fusion & creation of matter of the individual atom/molecule size is the core of nanotechnology. Nano assemblers are tiny devices which can have specific functions programmed into them by a computer. Nano assemblers, which may be minute than a cell nucleus, are made to fit in those areas that are beyond normal human naked eye vision or other such technology.

3 Biomarkers in Oral Cancer

Early identification of oral cancer reduces treatment costs and adverse effects while enhancing quality of life. The prognosis of oral cancer is heavily based on early identification due to a high probability of recurrence [20]. Despite recent advancements in this field, the nature of OSCC remains a considerable challenge. For the diagnosis of both primary and recurring oral cancer, levels of biological markers monitoring having high specificity and sensitivity is a futuristic diagnostic technique. According to the National Institute of Health, biomarkers are accurately and consistently measured indicators of healthy or diseased conditions [21]. Biomarkers include agents like mRNA, mutated DNA, secreted proteins, metabolomes along with other smaller molecules [22, 23] (Fig. 1b). A chemical released by a tumor or created in reaction to the beginning or development of cancer is identified as a cancer biomarker. A minimally invasive technique with high-positive and high-negative predictive values indicative of cancer stage should also be an element of the ideal biomarker. Such a biomarker can be utilized to forecast efficacy of the therapy, diagnosis as well as prognosis.

3.1 Oral Cancer Salivary Biomarkers

Collection of the secreted saliva is a promising biomarker tool since it is simple & painless. Previous research has examined the alterations in the salivary genome and protein composition subsequent to the initiation & progression of oral cancer [24, 25]. However, some acquaintances should be considered when gathering, processing and storing saliva. Biomarkers cannot be extrapolated into clinical applications without first undergoing cross-validation. Studies of oral cancer salivary biomarkers have produced wide variety of results, which could be explained by differences in data collecting, processing, and analysis methods. Therefore, more research is needed to standardize the techniques mentioned earlier along with reference values in order to obtain significance of such biomarkers.

3.2 RNA-Based Salivary Biomarkers

The primary function of salivary RNAases is break down RNA in the oral cavity. However, these RNAases biomarkers are not useful since they are either actively released from cellular vesicles like exosomes or transported in apoptotic bodies. Oral cancer patients have been reported to have significantly greater amount of miRNA 31 but lower levels of miRNA 200a & miRNA 125a. Malignant oral cancer has also been associated with downregulation of miRNA 145 and upregulation of miRNA 184 [26].

3.3 Protein-Based Salivary Biomarkers

Oxidative stress monitoring has the potential to be used as a biomarker for cancer detection. Cell toxicity results from irreversible protein degradation brought about by carbonylation. Salivary carbonyls are significantly raised in oral cancer due to reactive radical infiltration into the oral epithelium. Matrix metalloproteinases (MMPs) break down a variety of proteins. There has been evidence of considerable alterations to many types of MMPs during the extremely invasive and metastatic OSCC.

A poor prognosis for patients with oral cancer has been associated with MMP-2 and MMP-9 expression. Interleukins (IL) are proteins that are produced by the immune system. Naturally occurring proteins have a number of functions in cellular signaling cascades, some of which are essential in cancer. Two distinct forms of interleukins have been reported in increased OSCC prevalence: interleukin-6 (IL-6) & interleukin-8 (IL-8) [27].

3.4 Serum Biomarkers

The molecules produced during tumor growth are released into the blood, increasing serum biomarker levels that can be used to predict the spread of cancer. Biomarkers associated with specific tumors have recognized secretory sources. Regular monitoring of these indicators may provide valuable insights into patients' long-term responses to anticancer treatments.

Bloodstream contains the protein hormone adiponectin, which has several distinct actions. It facilitates the bloodstream's ability to absorb fatty acids and glucose. Reduced adiponectin levels have been linked to an aggressive tongue squamous cell carcinoma (TSCC) phenotype, according to research by Guo et al. [28]. Certain studies have also linked hypoadiponectinemia to lymphoma. For metastases from TSCC, the prognosis is dismal.

Anemia & low hemoglobin have been related to lymph node metastasis and enhanced tumor oxygenation, and raising hemoglobin levels has been demonstrated to improve cancer patients' prognosis. Advanced OSCC is indicated by low hemoglobin levels, particularly in more expansive tumors that need more radial therapy [29].

The function of pro-inflammatory cytokines like IL-6, IL-8, Soluble IL-2 receptor (SIL-2R), MHC class I polypeptide-related sequence B (MICB) & Tumor necrosis factor- alpha (TNF-α) in OSCC was examined by Schiegnitz et al. [30]. Their results indicated that serum samples from patients with oral cancer displayed an increased tendency for all of these indicators. The prognostic markers for OSCC that can be detected significantly are SIL-2R and IL-6. Chang et al. also discovered an association between pretreatment IL-6 serum levels and survival for OSCC patients [31]. However, because of these contradictions and the inconsistent literature searches, the results of such serum cytokines are not suitable candidates for oral cancer biomarkers. Nonetheless, some immune system and inflammatory system-related proteins may be self-contained. It has been suggested that patients with oral cancer who have elevated serum CRP may have poor prognosis & a low survival rate [32]. Vascular endothelial growth factor (VEGF) is hypothesized to stimulate angiogenesis in numerous tumor proliferation mechanisms. Elevations of this factor have been associated with advanced OSCC and nodal metastases. According to Schiegnitz et al., IGF-binding protein-3 and insulin-like growth factor-1 (IGF-1) are essential to the pathophysiology of OSCC and has potential to be utilised for prognosis [33].

4 Nano Diagnostics

Nano-diagnostics is the application of nanotechnology concepts to clinical diagnostics to meet the needs of early identification of diseases and higher sensitivity [34]. The diagnosis and treatment of cancer have been transformed by nanotechnology. It can identify a single malignant cell *in vivo* & target the diseased cells directly with highly toxic pharmaceuticals. Materials utilized for cancer detection include nanoshells, nano-tubes, quantum dots, super magnetic NPs, nano-wires, nano-diamonds, nano-sponges etc. [35]. Specific cancer cell crosslinkers, such as antibodies, can be used to identify particular cancer cells. Targeted, lipid-coated quantum dots present a unique array that might be used to evaluate several different indicators on the surface of particular cancer cells [35].

Various Nano diagnostic procedures and techniques involves:

- Nanoscale cantilevers: Elastic beams that bind to molecules linked to cancer [36]
- Cantilever array sensors: Technology of ultrasensitive mass detection [37]
- Nanopores: Allow single strand DNA to pass through hole one at a time, resulting in highly efficient DNA sequencing
- Nanotubes: Carbon rods with the ability to recognize mutated genes and specify their location

- Quantum dots: Localize tumors by attaching to proteins linked to cancer cells and glowing extremely brightly in ultraviolet light [36]
- Nanoelectromechanical System: Convert biochemical signals into electric signal
- Multiplexing modality: Concurrent sensing of several distinct biomolecules [37]

NPs have the potential to improve diagnosis accuracy, early disease identification, therapy progress, tumor burden tracking over time, and the degradation of exclusively targeted cancer cells due to their greater capacity to specifically target cancer cells including biomarkers. When plasmonic NPs are coupled to nuclear targeting peptides, cancer cells experience DNA damage & resultant apoptosis [38]. These NPs minimally harm normal tissues by selectively targeting malignant cells. An in-depth knowledge of tumor & cell biology along with immunology is required for sound rational designing of NPs. The advancement of cancer biology will have a significant impact on the development of nanotechnology methods [35].

4.1 Nanomaterials Used for Oral Cancer Diagnostics

Most effective treatment for cancer is prevention. Survival rates will increase significantly with early diagnosis; as it is easy to remove an in-situ tumor than a metastasized one. For gene and drug delivery as well as DNA structural probing, a variety of NP types are appropriate [39]:

- Solid lipid particles & Liposomes
- Polymeric nanoparticles, such as nanospheres and nano-capsules
- Nanoscale crystals
- Polymer therapeutics, such as fullerenes and dendrimers.
- Inorganic NPs, such as magnetic and gold nanoparticles.

Utilizing a reflectance-based optical imaging system, Kah et al. research [40] demonstrated how antibody-conjugated gold NPs may be utilized to both target and illuminate cancer cells. More specifically, they have demonstrated that when combined with antibodies, gold nanoparticles aid in the differentiation of cancerous from healthy cells and can also be utilized for tracking the expression of relevant biomarkers.

4.2 Tumor Bio-markers NPs

In Vitro Biomarker NPs

Tumor cell identification in vitro can be done qualitatively or quantitatively using NPs. By concentrating and shielding a marker from deterioration, they aid in the detection and increasing the sensitivity. An alternative strategy involved encasing inorganic biomarkers in place of fluorescent organic markers. These chemicals are more sensitive and appropriate for qualitative and particularly quantitative detection because they were more photo-stable and unaffected by the intrinsic fluorescence in background released by cells and tissues [41].

Using gold NPs conjugated to DNA probes in oral cancer salivary samples *in vitro*, Han et al. suggested that S100 calcium-binding protein P mRNA could be a promising biomarker for oral cancer having a detection limit of 3 nM [42].

In Vivo **Biomarker NPs**

The NPs' physicochemical properties (size, surface charge, coating & stability) enable the marker to be concentrated and redirected at the desired location. Colloidal particles with labels may be employed as radio diagnostic agents. However, several non-labeled colloidal systems are still being studied as contrast agents for diagnostic techniques including nuclear MRI and computed tomography, several of these systems are already in use [41].

5 Nanomedicine for Oral Cancer Therapy

Recent literature has shown that oral carcinogenesis is responding well to newer therapeutic molecules like siRNAs and different active targeting ligands alongside traditional approaches. The period of tumor cell contact with drug is a critical factor to consider while developing drug delivery systems. Consequently, a mucoadhesive targeted drug delivery system that for sustained adherence has been designed for use in oral cavity. Improved drug-mucus interaction and improved outcomes can be obtained by extending the residence period of nanoparticles.

Nanoparticle delivery locally can be used for location-specific treatment and chemoprevention (Fig. 1c). In order to specifically target cancer cells and biomarkers, a variety of nanoparticle forms, such as magnetic, polymeric, gold NPs, nanodiamonds & liposomes, have been investigated in the literature for cancer therapy [5]. Using particular crosslinkers, like aptamers or antibodies, to target cancer cells can also help in early disease detection. Oral cancer can originate from any sub-site of the oral cavity proper and eventually spread through the lymphatic system to other parts of the body. Tumor-associated protein and ligands are two examples of the targeted agents that can be functionalized to create nanocarriers. Aptamers, antigens and antibodies have all demonstrated potential in enhancing the delivery of a particular target to cancer cells. This technique can employ one or more drugs in variety of immunotherapy regimes and/or targeted drug/gene delivery routes [43]. An important factor in advancing nanotechnology is the careful formulation of NPs for cancer treatment with an extensive knowledge of cancer cell biology, immunology and pathology. Nanotechnology is likely the only method that can be used for site-specific action without affecting normal cells or causing adverse consequences. The newest development in cancer treatment is cancer nanotechnology. By working on at least two major levels—giving a pharmaceutical agent new characteristic (such enhanced stability, altered pharmacokinetics, reduced toxicity) and directing NPs straight into the tumor—it offers great deal of hope for enhancing cancer treatments [44].

NPs have the potential to function as tailored & precise drug delivery systems, delivering therapeutic genes or chemotherapeutic chemicals directly into cancerous cells while sparing healthy cells. Direct drug administration to the target tissue requires lower dosages of toxic substances & hence better tolerability. Certain nanoscale delivery agents, including cross-linked liposomes, silica-coated micelles, ceramic NPs & dendrimers; can be directed against cancer cells. As a result, drugs will be less damaging to healthy tissue and more selective toward cancer cells. The cell surface receptor ligands and monoclonal antibodies help for precision attachment to the cancerous cells. The versatility of nanomedicine platforms and their capacity to logically construct NPs to go through successive biological barriers are added advantages. This section describes contemporary research in NP-based drug delivery for Oral cancer management.

First generation NPs passively accumulated in tumors through the enhanced permeability and retention effect (EPR), which permitthe passive extravasation of NPs, due to increased vascularity of tumors, which has greater endothelial fenestrations than normal endothelium [45, 46]. Second generation NPs containing components for stimulus-responsive release (i.e. hypoxia & pH sensitive) and active targeting (i.e. monoclonal antibodies) exhibits improved tumor targeting [47–49]. Despite many benefits of both generations none of the NPs can overcome the various biological hinderances to NP drug delivery and none of them were FDA approved [47, 48]. Third generation NPs comprise of Multi-stage Nano Vectors (MSVs) that are made up of stage-one mesoporous silicon particles (S1MPs) & stage-two NPs encased in nanopores. S1MPs can interact with the endothelium after intravenous administration, enabling the release of stage-two NPs into the tumor mass [48, 50–52].

6 Chemotherapy Based on Nanoparticles

Chemotherapy has proven effectiveness in advanced oral cancer, but there is little evidence to support its beneficial role as an induction therapy [52]. Furthermore, traditional chemotherapy effectiveness is restricted by acquired drug resistance as well as unfavorable side effects. Therefore, the production of NPs linked with chemotherapeutic agents such cisplatin (CDDP), 5-fluorouracil (5FU) and molecular targeted agents were described in various studies.

6.1 Cisplatin

Ligand-decorated Cisplatin-loaded PLGA-PEG/NR7 NPs with a cancer targeting effect were developed by Wang et al. [53]. Compared to non-targeted NPs and CDDP, targeted NPs showed increased cytotoxicity, enhanced uptake by oral cancer cells and effective apoptosis. On the other hand, compared to CDDP, polymeric micelles containing cisplatin (NC-6004) showed lesser cytotoxicity against human oral cancer cell lines [54]. Subcutaneous therapy in mouse models treated with NC-6004 showed less renal damage than those treated with CDDP.

6.2 5-Fluorouracil (5-FU)

The anti-tumor efficacy of monomeric self-assembled nucleoside NPs tagged with 5-FU (5-FU-SNNP) was assessed by Zhao et al. in different *in-vitro* & *in-vivo* models [55]. Normal human oral epithelial cells were used as controls in the *in vitro* investigation. SNNPs showed minimal cytotoxicity toward controls and oral cavity SCC. For a duration of 3 weeks, 5-FU-SNNPs were locally administered in a subcutaneous xenograft model simulating oral cancer. Nanoparticle formulation of 5-FU demonstrated better long-term impact on tumor cells compared to the free drug formulation. Additionally, employing 5-FU-SNNPs was shown to substantially decrease tumor growth and toxicity.

6.3 Doxorubicin

A liposomal version of the potent chemotherapeutic agent doxorubicin (Dox) called Doxil® has been introduced to mitigate its cardiotoxic effects [46]. Other NPs like PEGylated and non-PEGylated liposomes containing the plant stilbene resveratrol (Res) combined with dox were synthesized by Mohan et al. [56]. In HNSCC cells, the drug combination worked better than either of the drug's free and liposomal forms. When compared to other controls, the dual drug-loaded liposome group exhibited a considerable increase in both apoptosis and DNA damage. Abbasi et al. [57] studied stimuli-responsive ionic mesoporous silica NPs coated with methotrexate (MTX) and Dox in an artificial oral cancer rat model. Following 14 weeks administration of both free and nanoforms of Dox along with MTX, there was no discernible change in HER2 expression and intravenous delivery of MTX-Dox NPs found to be more successful than oral dosing in decreasing the expression of MMPs and VEGF.

7 Future Perspectives and Strategies

The vast majority of research employing single entity NPs with stimulus-responsive components and active targeting (i.e. 2nd generation NPs) for local or systemic Oral cancer management has made it possible to improve drug bioavailability, solubility, anti-tumor effects of chemotherapeutic agents, and reduced toxicity. Subcutaneous tumor xenograft mouse was employed as animal models for research. These models are reproducible, but they differ greatly from the actual human disease in several ways, including tumor site, microenvironment, vascular and metastatic development [58, 59]. Orthotopic models may work better with chemically induced oral cancer and direct insertion of carcinoma cells into oral cavity [59].

The exceptionally high mortality rate of OSCC has been attributed to metastases rather than the primary tumor itself, rendering metastatic oral cancer models more valuable for the development of novel therapies [60]. After 31 days, metastases in the mandible, lungs, liver, and bone were found by Bais et al. [59] using human metastatic cell lines implanted into the tongue. Using the highly invasive & metastatic human OSCC cell line (OSC-19 cells) implanted into the tongues of nude mice, Kawashiri et al. found lymphatic metastases in 28 days [61]. Only a single study evaluated anti-metastatic benefits of NP systems on an oral cancer metastatic model [62]. The use of nanomedicine platforms in clinical settings is now being investigated for its potential as a therapeutic strategy for several cancer types, including OSCC. The current state of nanomedicine research for oral cancer management is emphasized in this chapter. Encouragement should be provided to develop and employ more appropriate animal models of OSCC in order to better gauge benefits of NPs, particularly regarding metastasis (such as orthotopic, metastatic models). Additionally, it's important to evaluate the in vivo and in vitro effectiveness of increasingly advanced platforms (e.g. MSVs, iNPG) in Oral cancer. In fact, next-generation platforms have the potential to improve the advantages of NP-based drug delivery—such as decreasing toxicity and overcoming chemoresistance—while also addressing issues like metastasis.

8 Potential Health Hazards

Within the field of toxicology, nanoparticles present a unique challenge called nanotoxicology. Structures with dimensions down to the nanoscale have very distinct characteristics. There is a dearth of research on the toxicological hazards associated with using nanotechnology in healthcare settings. Normally, the kidneys and intestines eliminate the waste products of the breakdown of biodegradable substances. Nonetheless, research on nonbiodegradable nanoparticles suggests that they tend to concentrate in the liver and other organs. Further research is required because it is unclear what dosage or potential harm they could trigger [39].

9 Conclusion

The use of nanotechnology in medicine, especially in the diagnosis and therapy of oral cancer, has the potential to significantly alter the healthcare delivery. Preclinical phases are being attained by many of the NP-based early cancer detection and treatment methods. Nanotechnology applications in the treatment of oral cancer may eventually replace traditional techniques, which often include painful drugs, radiation, and biopsies. The morbidity and mortality of HNSCC are directly impacted by the ability to diagnose malignant disease promptly as practical, which permits early planning of treatment options for comprehensive patient care.

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Navigating Safety and Toxicity Challenges in Nanomedicine: Strategies, Assessment, and Mitigation

Rajesh Kumar, Sunil Dutt, Abhay Dev Tripathi, Anurag Kumar Singh, Vivek K. Chaturvedi, and Santosh Kumar Singh

1 Introduction

The burgeoning domain of nanomedicine, an interdisciplinary convergence of medicine and nanotechnology, harbors profound potential to revolutionize healthcare by facilitating precise diagnostics, personalized therapeutic regimens, and targeted pharmacotherapies [1]. Engineered nanoparticles, owing to their distinctive physicochemical attributes, offer novel avenues for drug delivery, imaging modalities, and therapeutic interventions. However, notwithstanding these paradigm-shifting advancements, apprehensions regarding the cytotoxicity and biocompatibility of nanomaterials persist as formidable impediments to realizing the full therapeutic potential of nanomedicine [2].

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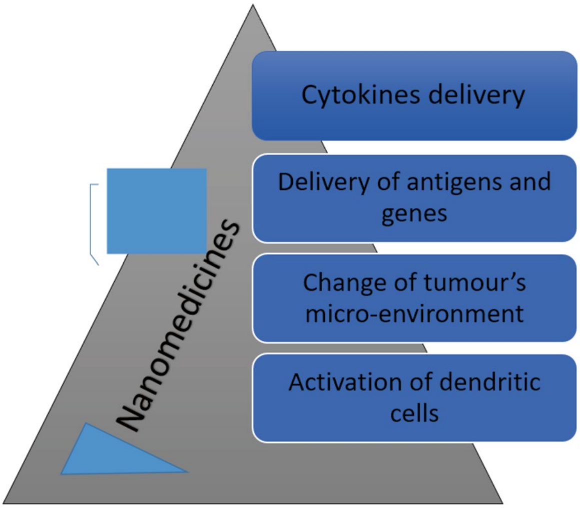
Fig. 1 Applications of nanomedicines in diverse area


Nanomedicines
Cytokines delivery
Delivery of antigens and genes
Change of tumour's micro-environment
Activation of dendritic cells

A comprehensive understanding of the safety profiles of nanoparticles is imperative, given the myriad challenges posed by their intricate interactions with biological milieu. The biological fate and potential toxicity of nanoparticles are contingent upon various factors, including their dimensions, morphology, surface functionalization, and degradability. Moreover, the assessment of nanoparticle safety and biocompatibility is further complicated by the diverse array of nanoparticle configurations and compositions [3].

This chapter endeavors to delineate strategies, assessment methodologies, and risk mitigation protocols aimed at holistically addressing the safety and toxicity concerns intrinsic to nanomedicine. Through an exploration of the multifaceted facets of nanoparticle-induced toxicity, encompassing oxidative stress, inflammatory responses, genotoxicity, and immunomodulation, we seek to provide comprehensive insights for academia, healthcare practitioners, regulatory bodies, and industry stakeholders [4]. Furthermore, we aim to scrutinize the plethora of *in vivo* and *in vitro* models employed in nanoparticle safety assessment, elucidating their merits, limitations, and translational relevance to human outcomes [5].

Additionally, we will delve into the regulatory frameworks governing the evaluation of nanomedicine safety, with a particular emphasis on pivotal parameters such as biocompatibility, pharmacokinetics, and biodistribution. Novel methodologies and analytical tools designed to augment the precision and efficacy of nanoparticle safety assessments will also be elucidated (Fig. 1).

Ultimately, this chapter aspires to furnish a thorough discourse on the safety and toxicity imperatives in nanomedicine, furnishing actionable guidance to mitigate risks and optimize the clinical utility of nanotechnological interventions. Effectively navigating these challenges holds the promise of harnessing the transformative potential of nanotechnology to optimize patient outcomes while ensuring compliance with regulatory standards [6].

2 Strategies for Safe Nanomedicine Development

2.1 Rational Design

At the core of safe nanomedicine advancement lies rational design, employing a systematic, knowledge-driven methodology to navigate the complexities of nanoparticle engineering. This approach hinges upon a methodical process commencing with precise delineation of treatment objectives and risk appraisal. Researchers are empowered to make informed decisions at each developmental stage by cultivating a comprehensive understanding of intended applications and associated hazards. Material selection assumes paramount importance in mitigating adverse effects, prioritizing biocompatible and biodegradable substrates [7]. Factors such as particle dimensions, surface charge, and surface functionalization are meticulously considered to ensure optimal drug delivery efficacy and enhance biocompatibility [6].

Critical to assessing the safety and efficacy of nanomedicines is preclinical evaluation. Rigorous investigations scrutinize pharmacokinetics, biodistribution, and toxicity profiles in relevant animal models, furnishing indispensable insights into potential hazards and benefits. Implementation of quality control protocols ensures consistency and purity of nanoparticle formulations, curbing batch-to-batch variability and ensuring product reliability. Surface engineering strategies are deployed to optimize nanoparticle stability, targeted specificity, and biocompatibility, enabling personalized drug delivery systems that minimize non-specific interactions [8].

Characterization studies, both *in vitro* and *in vivo*, yield further insights into nanoparticle behavior within biological milieus, encompassing cellular uptake, intracellular trafficking, and interactions with host tissues. Assessment of immunogenicity is pivotal for anticipating immune responses and mitigating immunotoxic risks, driving refinement of nanoparticle designs. As nanomedicines progress to clinical trials, meticulous attention is accorded to trial design, patient stratification, and endpoint delineation to furnish robust clinical evidence substantiating safety and efficacy [9].

Integral to nanomedicine development is regulatory adherence, ensuring the integrity of regulatory processes through stringent compliance with stipulated criteria. Comprehensive data pertaining to quality, safety, and efficacy is amassed for regulatory submissions, expediting assessment and approval procedures. Post-market surveillance mechanisms are employed to monitor the long-term efficacy and safety of nanomedicines in real-world clinical settings, facilitating ongoing evaluations of product effectiveness and patient outcomes [9].

2.2 Biocompatible Materials

The advancement of safe nanomedicine pivots on the utilization of biocompatible materials, offering a versatile scaffold for the fabrication of nanoparticles personalized for biological applications. Wide range of natural and biocompatible metal-based nanoparticles are designed and tested for their anticancer activity [7]. Central to this endeavor is the imperative to engineer materials that seamlessly integrate with biological systems to mitigate adverse effects and enhance therapeutic efficacy. Diverse biocompatible materials, encompassing lipids, inorganic nanoparticles, and organic polymers, have emerged as promising candidates for nanoparticle development, each endowed with unique benefits and attributes [9] (Fig. 2).

Natural polymers endowed with tunable physicochemical properties, such as chitosan, poly (lactic-co-glycolic acid) (PLGA), and polyethylene glycol (PEG), have garnered considerable attention owing to their innate biocompatibility and biodegradability. These polymers afford precise control over drug release kinetics and biodistribution, facilitated by facile functionalization enabling drug encapsulation, surface modification, and targeted delivery. Their favorable immunogenicity and toxicity profiles render them amenable for clinical translation, fostering the fabrication of diverse polymeric nanomedicines for drug delivery, imaging, and diagnostics [10].

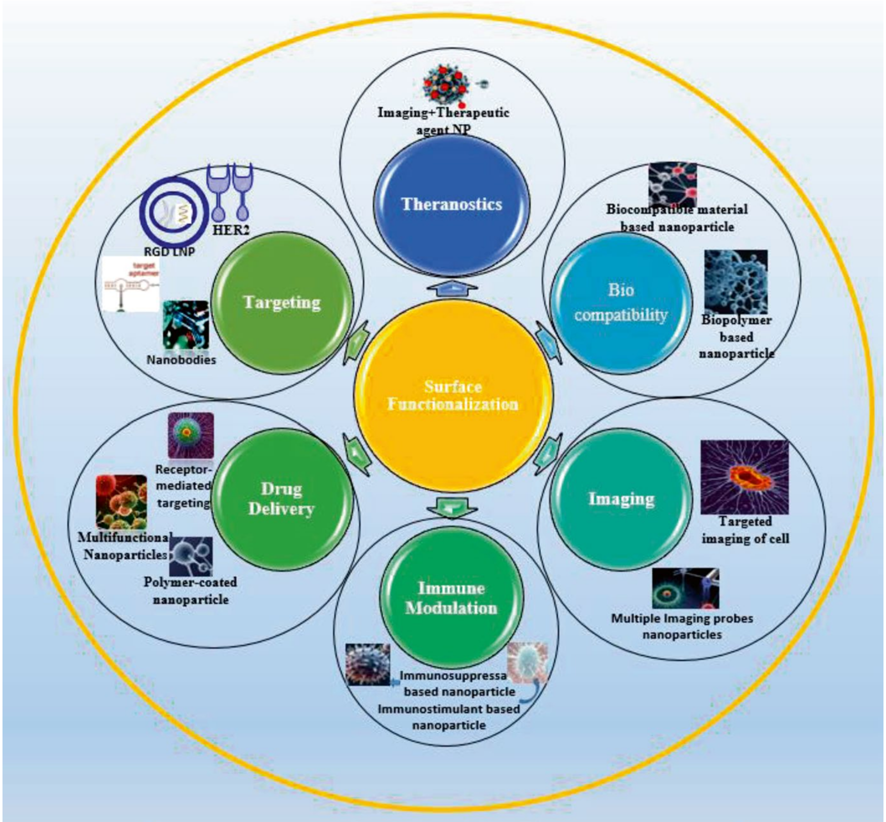

Imaging+Therapeutic agent NP
Theranostics
HER2
RGD LNP
target aptamer
Targeting
Nanobodies
Biocompatible material based nanoparticle
Bio compatibility
Biopolymer based nanoparticle
Surface Functionalization
Receptor-mediated targeting
Drug Delivery
Multifunctional Nanoparticles
Polymer-coated nanoparticle
Imaging
Targeted imaging of cell
Multiple Imaging probes nanoparticles
Immune Modulation
Immunosuppressant based nanoparticle
Immunostimulant based nanoparticle

Fig. 2 Strategies for safe nanomedicine development

Lipid-based nanoparticles represent another cohort of biocompatible materials with considerable therapeutic potential, encompassing liposomes, lipid nanoparticles, and solid lipid nanoparticles. Mimicking the natural lipid bilayers present in cellular membranes, these nanoparticles exhibit high biocompatibility and degradability, ensuring compatibility with biological milieu. Notably versatile, lipid nanoparticles encapsulate both hydrophilic and hydrophobic pharmaceuticals within their lipid bilayers or cores, enabling controlled drug release and enhanced therapeutic efficacy. Their appeal as safe and efficacious drug delivery vectors is further underscored by their ability to evade immune recognition and selectively target diseased tissues [11].

Inorganic nanoparticles, characterized by unique optical, magnetic, and mechanical properties, including gold, iron oxide, and silica, emerge as desirable candidates for diverse biomedical applications. While certain inorganic nanoparticles may exhibit intrinsic cytotoxicity, judicious surface engineering and functionalization strategies can mitigate risks and enhance biocompatibility. Surface modifications with biocompatible coatings like PEG or silica shells shield nanoparticles from immune surveillance and facilitate targeted delivery, ensuring their safe integration into biological systems [12].

Leveraging insights from materials science, chemistry, biology, and medicine, a multidisciplinary approach underpins the development of biocompatible materials for safe nanomedicine. Employing systematic design and meticulous optimization, scientists tailor nanoparticle characteristics to address specific therapeutic requisites while mitigating cytotoxicity, immunogenicity, and off-target effects [13].

2.3 Surface Functionalization

Surface functionalization plays a pivotal role in enhancing biocompatibility, targeted specificity, and therapeutic efficacy while mitigating potential risks associated with nanoparticle and nanozyme interactions within biological systems [14]. Essentially, surface functionalization entails modifying the surfaces of nanoparticles through the incorporation of biologically active molecules, polymers, or coatings to tailor their physicochemical properties and biological interactions [15].

Primarily, surface functionalization aims to diminish immunogenicity and augment biocompatibility by evading immune recognition of nanoparticles. Polyethylene glycol (PEG), owing to its hydrophilicity and ability to establish a steric barrier hindering protein adsorption and opsonization, stands out as one of the most widely employed surface modifiers. This prolongs nanoparticle circulation time and reduces clearance by the reticuloendothelial system (RES), thereby enhancing their safety profile as they clandestinely navigate the circulation to reach target sites without eliciting immune detection and clearance [16].

Furthermore, surface functionalization facilitates precise modulation of nanoparticle interactions with target cells or tissues, thereby minimizing off-target effects and enabling targeted drug delivery. Surface functionalization with ligands such as peptides, antibodies, or small molecules confers targeted selectivity toward diseased cells or tissues expressing specific biomarkers. This personalized approach not only enhances therapeutic efficacy but also minimizes systemic exposure, mitigating adverse effects on healthy tissues by delivering medications directly to the site of action [17].

Moreover, surface functionalization can be harnessed to regulate drug release kinetics, ensuring sustained therapeutic benefits. Incorporation of stimuli-responsive moieties or cleavable linkers onto nanoparticle surfaces enables drug release triggered by environmental cues such as pH, temperature, or enzyme activity. This spatiotemporal control over drug release optimizes therapeutic efficacy at the target site while minimizing systemic exposure to high drug concentrations, thereby lowering the risk of systemic toxicity [18].

Additionally, surface functionalization strategies can be personalized to address specific challenges associated with nanoparticle-mediated drug delivery, including intracellular trafficking and cellular uptake. Surface-functionalized nanoparticles engineered with cell-penetrating peptides or endosomal escape mechanisms effectively ferry medications to intracellular targets and surmount biological barriers, maximizing nanoparticle therapeutic potential while attenuating dose-dependent toxicity [19].

2.4 Targeted Delivery

An integral facet of safe nanomedicine advancement lies in targeted delivery, offering a sophisticated approach to maximize therapeutic efficacy while mitigating off-target effects and systemic toxicity. Targeted delivery, in essence, entails the precise localization of medicinal agents to specific cells, tissues, or organs guided by biological pathways or molecular markers intricately linked to the pathophysiology of a disease. This personalized strategy harbors the potential to circumvent many limitations associated with conventional systemic administration routes, thereby revolutionizing drug delivery paradigms [20].

Targeted delivery confers several advantages, foremost among which is its ability to minimize systemic exposure to therapeutic agents, thereby reducing the risk of adverse effects on healthy tissues and organs [21]. Leveraging molecular targeting ligands, encompassing peptides, antibodies, and small molecules, nanoparticles can be engineered to selectively bind to receptors or antigens overexpressed on diseased cells or tissues. By precisely delivering medications to the site of action, this personalized approach maximizes drug accumulation within diseased tissues while minimizing exposure to adjacent healthy tissues. Consequently, by mitigating off-target effects, targeted administration not only enhances therapeutic efficacy but also augments the safety profile of nanomedicines [22].

Moreover, personalized delivery facilitates traversing biological barriers and augmenting intracellular distribution of therapeutic agents to their intended targets. Nanoparticles adorned with cell-penetrating peptides or endosomal escape mechanisms enable more efficient delivery of therapeutics to intracellular compartments where they exert their pharmacological effects. These modifications enhance cellular uptake and intracellular trafficking, thereby not only enhancing therapeutic efficacy but also enabling targeting of intracellular pathways and processes inaccessible to traditional drug delivery modalities [23].

Furthermore, by circumventing efflux pathways and delivering therapeutic compounds directly to cancer cells, targeted delivery represents a promising strategy for overcoming multidrug resistance, a formidable obstacle in cancer therapy. Targeted nanomedicines can bypass the resistance mechanisms that often curtail the efficacy of conventional chemotherapy regimens by selectively targeting cancer cells while sparing normal cells. This opens new vistas for enhancing treatment outcomes and improving patient survival rates in oncological settings [24].

2.5 Drug Loading and Release

The development of safe nanomedicine hinges on the implementation of effective drug loading and release strategies aimed at maximizing therapeutic efficacy while minimizing off-target effects and systemic toxicity. These strategies afford precise control over drug delivery kinetics, encompassing drug loading, which involves the incorporation of therapeutic agents such as proteins, nucleic acids, imaging agents, or small molecules into nanoparticle carriers, and drug release, which entails the gradual release of these agents at the designated site. Several variables, including the properties of the drug, nanoparticle characteristics, and environmental cues, intricately influence these processes and can be meticulously personalized to optimize therapeutic outcomes [25].

Achieving optimal encapsulation efficiency of therapeutic agents within nanoparticle carriers is paramount in drug loading, ensuring effective drug delivery to the target site while mitigating the risk of premature release and systemic exposure. Various loading techniques, such as chemical conjugation, physical encapsulation, and electrostatic interaction, can be employed to attain high drug loading capacities and encapsulation efficiencies. Through judicious selection of loading techniques and formulation optimization, scientists can customize drug loading to meet specific therapeutic requirements, including targeted release kinetics and payload masses [26].

Precise modulation of drug release kinetics is imperative for maximizing therapeutic efficacy and minimizing systemic toxicity and off-target effects. Controlled release mechanisms, including stimuli-responsive release, degradation-controlled release, and diffusion-controlled release, afford spatiotemporal control over drug release, ensuring sustained therapeutic concentrations at the target site while minimizing fluctuations in systemic drug levels. For instance, stimuli-responsive nanocarriers can be engineered to respond to specific environmental cues, such as pH, temperature, or enzyme activity, selectively releasing the drug only under disease-specific conditions [27].

Furthermore, customized drug release techniques can overcome biological barriers and enhance intracellular drug delivery to target sites. Incorporating endosomal escape or intracellular trafficking pathways into nanoparticles designed for intracellular drug delivery facilitates efficient release of drugs into the cytoplasm or specific organelles, where they exert their pharmacological effects. Besides augmenting treatment efficacy, this focused intracellular delivery minimizes exposure of cytotoxic agents to healthy tissues, thereby reducing the risk of off-target effects and systemic toxicity [28].

3 Assessment Methods for Safety Evaluation

3.1 Physicochemical Characterization

The foundational assessment of nanomedicine progress relies on physicochemical characterization, furnishing crucial insights into the morphological, structural, and physicochemical attributes of nanoparticles governing their interactions with biological systems. This multifaceted approach aims to elucidate nanoparticle properties including size, shape, surface charge, composition, stability, and drug loading capacity through a diverse array of methodologies and techniques. Systematic characterization empowers researchers to anticipate nanoparticle behavior in biological milieus, refine their design, and assess their safety and efficacy for clinical translation [29].

Nanoparticle size stands as a pivotal factor in physicochemical characterization, exerting profound effects on pharmacokinetics, biodistribution, and cellular uptake. Techniques such as transmission electron microscopy (TEM) and dynamic light scattering (DLS) are commonly employed to scrutinize nanoparticle size and morphology, furnishing critical insights into particle shape, size distribution, and uniformity [30]. Optimization of particle size is imperative to ensure effective drug delivery and cellular internalization while mitigating potential risks associated with particle aggregation and immune recognition [31].

Surface charge represents another critical determinant affecting biodistribution, cellular interactions, and nanoparticle stability. Quantification of nanoparticle surface charge is often achieved through zeta potential analysis, where positive or negative zeta potentials signify surface charge characteristics that can influence nanoparticle behavior in biological settings. Optimization of surface charge is indispensable for mitigating nonspecific bio-molecular interactions, enhancing colloidal stability, and enabling targeted delivery via electrostatic interactions with biological receptors or cell membranes [32].

Furthermore, composition and surface chemistry play pivotal roles in physicochemical characterization, dictating drug loading capacity, functionalization potential, and biocompatibility of nanoparticles. Methods such as X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FTIR) enable researchers to assess material purity, identify functional groups, and ascertain surface modifications, furnishing valuable insights into nanoparticle composition and surface chemistry. Precise modulation of surface functionalization empowers researchers to devise targeted delivery systems, enhance biocompatibility, and optimize drug loading efficiency by tailoring nanoparticle composition and surface chemistry [33].

Moreover, physicochemical characterization encompasses evaluation of drug loading capacity and particle stability to ensure uniformity and reproducibility in nanoparticle formulations. Techniques including thermogravimetric analysis (TGA), fluorescence spectroscopy, and UV-visible spectroscopy facilitate measurement of pharmacological loading efficiency, encapsulation efficiency, and stability under various environmental conditions. These parameters are instrumental in assessing the scalability of production methods, optimizing drug delivery kinetics, and forecasting nanoparticle behavior in biological systems [16] (Fig. 3).

3.2 In Vitro *Studies*

Before advancing to more complex *in vivo* models, *in vitro* studies serve as a controlled and reproducible platform for evaluating the behavior, efficacy, and safety of nanoparticle formulations, constituting a critical component of nanomedicine

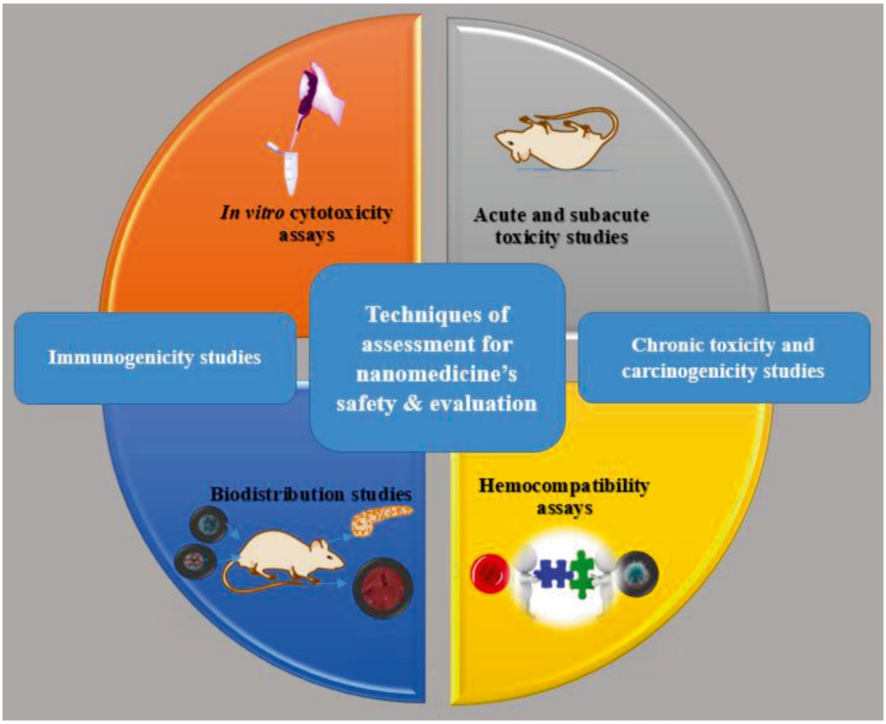

In vitro cytotoxicity assays
Acute and subacute toxicity studies
Immunogenicity studies
Techniques of assessment for nanomedicine's safety & evaluation
Chronic toxicity and carcinogenicity studies
Biodistribution studies
Hemocompatibility assays

Fig. 3 Assessment methods for safety evaluation of nanomedicines

development assessment. These investigations encompass a diverse array of experimental methodologies and assays aimed at elucidating various aspects of nanoparticle behavior, including cytotoxicity, intracellular trafficking, cellular uptake, and therapeutic efficacy. Systematic execution of *in vitro* investigations enables researchers to glean insights into nanoparticle-cell interactions, refine formulation parameters, and identify candidates for further preclinical and clinical evaluation [34].

Central to *in vitro* research is the evaluation of cellular uptake and intracellular fate of nanoparticle formulations, elucidating their mechanisms of action and potential for targeted drug delivery. Cellular uptake assays, employing techniques such as flow cytometry, confocal microscopy, and quantitative fluorescence microscopy, facilitate visualization and quantification of nanoparticle internalization by target cells over time. Researchers can optimize nanoparticle designs to enhance therapeutic efficacy while minimizing off-target effects by assessing parameters such as particle size, surface charge, and surface functionalization, thereby improving cellular uptake efficiency and intracellular trafficking [35].

Moreover, in vitro investigations enable assessment of nanoparticle cytotoxicity and biocompatibility critical considerations for evaluating safety profiles and clinical translatability. Cell viability assays, including the MTT assay and live/dead staining assays, afford researchers the ability to quantify cell viability and discern potential adverse effects of nanoparticle formulations on both target and non-target cells. Systematic examination of cytotoxicity in relevant cell models allows detection of potential hazards associated with nanoparticle exposure, prompting modification of formulation parameters to mitigate adverse effects [36].

In addition to cytotoxicity and cellular uptake analysis, *in vitro* investigations are instrumental in determining the therapeutic efficacy of nanoparticle formulations. Measurement of therapeutic compound release from nanoparticle carriers under physiological conditions can be achieved through drug release assays, encompassing drug release kinetics studies and release profiling tests. Optimization of nanoparticle formulations, considering factors such as drug loading efficiency, release kinetics, and therapeutic concentrations, enables researchers to maximize therapeutic outcomes while minimizing systemic toxicity and off-target effects, thereby achieving desirable therapeutic efficacy [37].

3.3 In Vivo

An indispensable stage in assessing the advancement of nanomedicine involves conducting *in vivo* investigations, which furnish critical insights into the pharmacokinetics, biodistribution, safety, and efficacy of nanoparticle formulations in living organisms. By administering nanoparticle formulations to animal models, researchers gain valuable information regarding their behavior, therapeutic benefits, and potential toxicities within complex biological systems, thereby bridging the gap between preclinical research and clinical translation. Methodical execution of *in* *vivo* experiments guides the optimization and validation of nanoparticle formulations for medicinal applications [38].

A primary objective of *in vivo* research is to assess the pharmacokinetics and biodistribution of nanoparticle formulations post-injection. Researchers quantify the concentration of therapeutic drugs and nanoparticles in systemic circulation and various organs/tissues over time using techniques such as tissue homogenization, positron emission tomography, and imaging modalities. Evaluation of nanoparticle designs aims to maximize circulation time, biodistribution, and accumulation at target sites while minimizing nonspecific uptake and clearance by the reticuloendothelial system, with considerations including nanoparticle size, surface charge, and surface functionalization [39].

Furthermore, *in vivo* investigations facilitate evaluation of the therapeutic efficacy of nanoparticle formulations in relevant disease models [40]. Utilizing animal models such as tumor xenografts, inflammatory models, and infection models, scientists assess the ability of nanoparticle compositions to target diseased tissues, deliver therapeutic drugs, and elicit desirable biological responses. Measurement of endpoints such as tumor development prevention, inflammation reduction, or pathogen clearance enables assessment of the therapeutic potential of nanoparticle formulations, driving improvements in design to enhance efficacy and clinical relevance [41].

In addition to therapeutic effectiveness, *in vivo* investigations enable evaluation of nanoparticle safety profiles and potential toxicities. Examination of organ function, serum biomarker levels, and histopathological analysis elucidates potential adverse effects on organ structure and function following nanoparticle injection. Systematic assessment of *in vivo* toxicity allows researchers to identify potential hazards associated with nanoparticle exposure, optimize formulation parameters, and mitigate unfavorable outcomes to ensure the safety of future clinical applications [42].

3.4 Immunotoxicity Evaluation

An integral aspect of comprehensive nanomedicine development assessment is the evaluation of immunotoxicity, focusing on potential adverse effects of nanoparticle formulations on the immune system. The immune responses triggered by nanoparticles upon interaction with biological systems can impact their safety, efficacy, and clinical translation [43]. Immunotoxicity assessment employs a range of assays and methodologies, including cytokine analysis, immune cell profiling, complement activation tests, and histopathological examination, to elucidate the immunological impacts of nanoparticles. Through systematic evaluation of immunotoxicity, researchers can identify potential hazards associated with nanoparticle exposure and optimize formulation parameters to mitigate unfavorable immune responses [44].

A primary concern in immunotoxicity assessment is the possibility of nanoparticles inducing inflammatory reactions and cytokine production, leading to detrimental systemic effects and immune-mediated toxicity. Cytokine analysis allows researchers to quantify pro- and anti-inflammatory cytokine levels in serum or tissue samples post nanoparticle exposure, utilizing techniques such as enzyme-linked immunosorbent assays (ELISA) or multiplex assays. Monitoring changes in cytokine profiles enables assessment of nanoparticles immunomodulatory effects and identification of immune-related adverse events [45].

Additionally, evaluating nanoparticles effects on immune cell populations and functions, including phagocytic activity, T cell activation, and dendritic cell maturation, is crucial in immunotoxicity assessment. Immunohistochemistry, flow cytometry, and functional assays enable characterization of immune cell subsets responses to nanoparticle exposure, facilitating detection of potential immunosuppressive or immunostimulatory effects and elucidation of underlying mechanisms [46].

Assessment of complement activation is another key aspect of immunotoxicity evaluation, as it represents an integral component of the innate immune response that may be triggered by nanoparticle exposure. Complement activation assays, such as complement deposition assays or hemolytic assays, enable measurement of complement activation products and evaluation of nanoparticles potential to induce complement activation. Assessment of complement activation levels aids in identifying potential risks of inflammatory reactions and complement-mediated damage associated with nanoparticle exposure [24].

Furthermore, histopathological examination of immune-related organs and tissues, such as the spleen, lymph nodes, and bone marrow, provides valuable insights into potential histological alterations suggestive of immune-mediated toxicity. Histopathological analysis guides interpretation of immunotoxicity data and informs safety evaluations by identifying tissue damage, inflammation, and immune cell infiltration following nanoparticle exposure [24].

3.5 Long-Term Safety Studies

Long-term safety studies are indispensable for a comprehensive understanding of the evolution of nanomedicine, as they provide crucial insights into the potential benefits and risks associated with prolonged nanoparticle exposure. While short-term studies offer initial safety assessments, long-term investigations are essential for elucidating cumulative effects, chronic toxicity, and possible adverse events that may manifest over extended periods. Through systematic long-term safety studies, researchers can evaluate nanoparticle formulation stability, identify delayed toxicities, and ensure the safety and efficacy of nanomedicines for clinical translation [2].

A primary objective of long-term safety studies is the assessment of systemic toxicity and biodistribution of nanoparticles following prolonged exposure. Commonly utilized animal models such as non-human primates and rats are employed to evaluate the long-term effects of nanoparticle administration on organ function, tissue integrity, and overall health. Systematic monitoring of physiological parameters including food intake, body weight, organ function tests, and hematological markers enables researchers to assess animal's general health status over time and detect potential indicators of systemic toxicity. Longitudinal imaging studies such as positron emission tomography (PET) and magnetic resonance imaging (MRI) facilitate the evaluation of nanoparticle biodistribution and clearance from systemic circulation and various organs/tissues, aiding in the assessment of potential accumulation and persistence *in vivo* [24].

Furthermore, long-term safety studies encompass evaluation of potential genotoxicity and carcinogenicity associated with nanoparticle exposure. Genotoxicity assessments utilizing assays such as the comet or micronucleus assays enable evaluation of chromosomal abnormalities and DNA damage induced by nanoparticles in cells or tissues following extended exposure. Long-term carcinogenicity studies involve nanoparticle administration to animal models and systematic monitoring of tumor occurrence and progression. By assessing genotoxicity and carcinogenicity in long-term studies, researchers can evaluate potential risks associated with nanoparticle exposure and identify any indications warranting further investigation [47].

Additionally, long-term safety studies provide an opportunity to evaluate potential immunological effects and immune responses elicited by nanoparticles over time. Longitudinal profiling of immune cell populations, cytokine levels, and immune function tests enables researchers to monitor changes in the immune system and assess immunomodulatory effects associated with nanoparticle exposure. Systematic evaluation of immunological parameters in extended research investigations allows for the identification of potential risks of immune-mediated toxicity and informs safety assessments for clinical applications [48].

4 Mitigation Strategies for Toxicity Management

4.1 Surface Modification

Surface modification plays a pivotal role in developing strategies to mitigate toxicity in nanomedicine by offering a versatile approach to enhancing biocompatibility, mitigating immunogenicity, and minimizing adverse outcomes associated with nanoparticle exposure. The surface properties of nanoparticles are critical in dictating their interactions with biological systems, encompassing cells, tissues, and physiological environments. Through the incorporation of biocompatible coatings, targeted ligands, or stealth compounds onto nanoparticle surfaces, surface modification techniques can enhance their safety profile for clinical applications while diminishing off-target effects [46].

One of the primary objectives of surface modification is to evade recognition by the immune system, thereby enhancing biocompatibility and reducing immunogenicity. Polyethylene glycol (PEG), renowned for its hydrophilicity and ability to establish a steric barrier that impedes protein adsorption and opsonization, is a frequently employed surface modifier. PEGylation extends circulation time and diminishes clearance by the reticuloendothelial system (RES), effectively managing toxicity in nanomedicine by enhancing biocompatibility, attenuating immune recognition, and diminishing the likelihood of immune-mediated adverse effects [49].

Furthermore, surface modification enables precise control over nanoparticle interactions with biological entities, facilitating targeted distribution and mitigating unintended consequences. Functionalization of nanoparticle surfaces with targeted ligands, such as peptides, antibodies, or small molecules, enables selective binding to receptors or antigens overexpressed on diseased cells or tissues. This personalized approach not only enhances therapeutic efficacy but also minimizes systemic exposure, thereby mitigating potential adverse effects on healthy tissues [49].

Moreover, surface modification techniques can enhance the biocompatibility and stability of nanoparticles, thereby reducing the risk of immunological recognition and cytotoxicity. Incorporation of biocompatible polymers or lipid coatings onto nanoparticles can improve their *in vivo* safety profile by preventing aggregation, enhancing colloidal stability, and reducing non-specific interactions with biological components. Additionally, surface modification using stealth molecules, such as zwitterionic polymers or carbohydrate-based coatings, can further enhance the biocompatibility and safety of nanomedicines by minimizing immunological recognition and prolonging circulation duration [50].

4.2 Dose Optimization

A pivotal strategy in toxicity control for nanomedicine is dose optimization, offering a systematic approach to mitigate side effects while enhancing therapeutic efficacy. Balancing the potential toxicity of nanoparticles with their therapeutic benefits within biological systems hinges on carefully calibrating nanoparticle dosages. By methodically optimizing nanoparticle doses, researchers can harness therapeutic advantages while minimizing the risk of systemic toxicity and off-target effects, thereby advancing the safety profile of nanomedicines for clinical applications.

At the conclusion of dose optimization lies the quest for the lowest effective dose that achieves therapeutic efficacy while minimizing side effects. Rigorous dose-ranging experiments in animal models are conducted to elucidate the dose-response relationship and pinpoint the optimal therapeutic window for nanoparticle formulations. By evaluating parameters such as drug concentration, nanoparticle dosage, and treatment duration, researchers discern dosages yielding maximal therapeutic benefits with minimal toxicity. This insight informs the design of optimal dosing schedules for clinical investigations [51].

Furthermore, dose optimization considers the pharmacokinetics and biodistribution of nanoparticles to ensure effective drug delivery while mitigating systemic exposure and off-target effects. Pharmacokinetic studies enable the assessment of drug and nanoparticle concentrations in various organs, tissues, and systemic circulation over time, shedding light on drug distribution, metabolism, excretion, and absorption dynamics. By tailoring nanoparticle formulations to enhance drug delivery efficiency and minimize systemic exposure, researchers can reduce the risk of systemic toxicity while maximizing therapeutic efficacy [52].

Moreover, dose optimization strategies can address specific challenges encountered with nanoparticle-mediated drug delivery, such as drug resistance, physiological barriers, and tumor heterogeneity. Modulating nanoparticle dosages based on tumor characteristics, including size, stage, and molecular features, ensures optimal drug distribution and treatment outcomes. Additionally, investigating combination therapies comprising synergistic drug combinations or multimodal approaches can enhance treatment efficacy and overcome resistance mechanisms, enabling lower individual drug doses and mitigating toxicity risks [53].

To further mitigate toxicity while preserving therapeutic efficacy, dose fractionation and timing strategies can be employed alongside dose optimization techniques. Fractionated dosing regimens involve administering lower doses over multiple cycles to minimize systemic exposure and potential toxicity while allowing for gradual accumulation of therapeutic agents at the target site. Similarly, incorporating drug-free intervals within dosage schedules can reduce the risk of cumulative toxicity and facilitate tissue regeneration between treatments [54].

4.3 Targeting Strategies

Targeting techniques play a crucial role in mitigating systemic toxicity and enhancing therapeutic efficacy in nanomedicine by providing precise and selective delivery of nanoparticles to specific cells, tissues, or organs. By minimizing off-target effects and reducing exposure to healthy tissues, these methods offer sophisticated strategies for improving the safety profile of nanomedicines. Various targeting approaches, including ligand-mediated targeting, stimuli-responsive targeting, and physicochemical targeting, each offer unique benefits for enhancing the safety and effectiveness of nanomedicines [53].

The primary objective of targeting techniques is to achieve targeted accumulation of nanoparticles at the site of disease while minimizing systemic exposure to healthy tissues. Ligand-mediated targeting involves conjugating targeting ligands, such as antibodies, peptides, or small molecules, to the surfaces of nanoparticles. This allows for precise binding to receptors or antigens that are overexpressed on diseased cells or tissues, facilitating the delivery of therapeutic drugs specifically to the site of action, thereby increasing therapeutic efficacy while reducing systemic toxicity and off-target effects [55].

Stimuli-responsive targeting techniques enable spatiotemporal control over drug release and nanoparticle accumulation in response to specific physiological or pathological signals. By incorporating stimuli-responsive moieties, such as temperature-responsive lipids, pH-sensitive polymers, or enzyme-cleavable linkers, therapeutic drugs can be released on-demand in response to changes in the intracellular environment or tumor microenvironment. This enhances drug delivery selectivity and accuracy, minimizing systemic exposure and off-target effects while optimizing therapeutic efficacy [56].

Physicochemical targeting techniques leverage the unique physicochemical properties of nanoparticles to promote selective accumulation at the site of disease. By exploiting features such as size, shape, or surface charge, nanoparticles can be designed to exploit physiological aspects of the tumor microenvironment, such as the enhanced permeability and retention (EPR) effect or tumor-specific cellular absorption mechanisms. This enables researchers to selectively target therapeutic drugs to tumors while minimizing exposure to healthy tissues, thereby reducing the risk of systemic toxicity [57].

In addition to enhancing therapeutic efficacy, targeting techniques can also be utilized to reduce systemic toxicity and potential off-target effects associated with nanoparticle exposure. By specifically targeting diseased tissues or cells with nanoparticles, the potential for harmful effects on normal physiological functioning can be minimized, while exposure to healthy tissues can be restricted. Combining targeted delivery systems with dose optimization techniques further allows for the administration of therapeutic drugs at lower dosages, thereby preserving therapeutic efficacy while minimizing the risk of systemic toxicity [58].

4.4 Combination Therapies

Combination medicines hold significant promise for mitigating toxicity and enhancing therapeutic outcomes in nanomedicine through their synergistic benefits, dose-sparing effects, and improved targeting strategies. By combining therapeutic agents with complementary modes of action, researchers can achieve enhanced efficacy while minimizing side effects and systemic toxicity.

One of the primary advantages of combination medicines is their ability to target multiple pathways or molecular targets associated with disease progression, thereby increasing therapeutic effectiveness and overcoming resistance mechanisms. Nanoparticles can be engineered to co-deliver multiple therapeutic agents, such as immunotherapies, chemotherapeutic drugs, targeted therapies, or nucleic acid-based therapeutics, to diseased tissues or cells. By simultaneously attacking multiple biological targets, combination therapies can reduce the risk of drug resistance and treatment failure while achieving superior treatment outcomes, such as tumor shrinkage, decreased metastasis, and prolonged survival [50].

Furthermore, combination medicines enable the use of individual drugs at lower doses, thereby reducing systemic exposure and potential toxicity while maintaining therapeutic efficacy. Co-delivering therapeutic agents within the same nanoparticle carrier allows for synergistic effects at lower doses, minimizing the risk of adverse effects and improving patient compliance and tolerance. This dose-sparing effect enhances treatment outcomes and quality of life while minimizing off-target effects and systemic toxicity associated with high drug doses [59].

In addition, combination medicines can capitalize on complementary modes of action to optimize drug delivery effectiveness and reduce side effects. Nanoparticles can be designed to co-deliver therapeutic agents with distinct physicochemical properties, such as hydrophobic and hydrophilic drugs, to achieve optimal drug release kinetics and tissue penetration. By optimizing nanoparticle formulations for improved tissue penetration and targeted drug delivery, researchers can achieve synergistic benefits with minimal exposure to healthy tissues and reduced side effects related to non-specific biodistribution [60].

Overall, combination medicines offer a promising approach to improving drug distribution, overcoming biological barriers, and maximizing therapeutic efficacy while minimizing toxicity in nanomedicine. Through the strategic combination of therapeutic agents and advanced targeting strategies, researchers can achieve superior treatment outcomes with reduced side effects, ultimately leading to improved patient outcomes and quality of life [61].

4.5 Regulatory Compliance

Regulatory compliance serves as a cornerstone in the validation of nanomedicine, ensuring meticulous adherence to stringent safety and efficacy standards prior to clinical deployment. Esteemed regulatory bodies such as the European Medicines Agency (EMA) in Europe and the Food and Drug Administration (FDA) in the United States demand comprehensive preclinical and clinical data to ascertain the safety, efficacy, and quality of nanoparticle-based treatments. Foremost among these mandates is the execution of exhaustive preclinical safety assessments to delineate potential risks associated with nanoparticle exposure. Rigorous evaluation of nanoparticle formulations encompasses scrutiny of physicochemical attributes, biodistribution profiles, pharmacokinetics, and toxicity profiles in preclinical animal models. By discerning potential toxicities and establishing dose-response relationships, preclinical investigations lay the groundwork for ensuring the safety of human subjects in subsequent clinical trials [62].

Furthermore, regulatory compliance necessitates the implementation of robust production and quality control protocols to uphold the consistency, stability, and purity of nanoparticle formulations. Adherence to Good Manufacturing Practice (GMP) guidelines is imperative for nanomedicine manufacturers, as these regulations dictate stringent production standards encompassing quality assurance, quality control, and process validation. By rigorously adhering to GMP principles, manufacturers mitigate batch-to-batch variability and uphold the safety and efficacy of nanoparticle formulations intended for clinical application [63].

Moreover, meticulously designed and adequately powered clinical trials are indispensable for scrutinizing the safety, efficacy, and tolerability of nanomedicines in human subjects. Clinical trials must rigorously adhere to predefined study protocols, ensure rigorous informed consent procedures, and institute ethical oversight mechanisms to safeguard the rights and well-being of trial participants. By methodically assessing nanomedicines in controlled clinical settings, researchers generate robust clinical evidence to underpin regulatory approval and inform clinical practice guidelines [64].

Lastly, comprehensive post-market surveillance is imperative to monitor the ongoing safety and effectiveness of nanomedicines subsequent to regulatory approval. Pharmacovigilance programs play a pivotal role in systematically capturing, analyzing, and reporting adverse events associated with nanomedicines. By establishing robust pharmacovigilance systems, regulatory agencies can promptly detect emerging safety concerns, update prescribing guidelines, and implement risk mitigation strategies, thus ensuring the sustained safety and efficacy of nanomedicines in clinical practice [65].

5 Challenges and Considerations

Formulations in nanomedicine hold promise for enhancing therapeutic efficacy, enabling precise drug delivery, and reducing systemic toxicity. However, realizing their full potential in clinical settings requires addressing a multitude of challenges spanning formulation design, production, regulatory compliance, and clinical translation. Tackling these issues demands interdisciplinary collaboration and innovative approaches, encompassing various aspects from manufacturing intricacies to regulatory hurdles [66].

The complexity inherent in designing and manufacturing nanoparticles poses a significant hurdle in nanomedicine development. Advanced engineering methodologies and scalable manufacturing processes are indispensable for crafting nanoparticles with precise dimensions, shapes, surfaces, and drug-loading capacities. Achieving repeatability and consistency in nanoparticle production, vital for regulatory approval and clinical translation, necessitates stringent quality control measures and standardized manufacturing protocols [67].

Moreover, ensuring the stability and biocompatibility of nanomedicine formulations presents inherent challenges due to their interactions with biological systems. Nanoparticles must maintain structural integrity and functionality post-delivery, resisting physiological degradation, opsonization, and aggregation. Addressing stability and biocompatibility concerns requires careful selection of materials, surface modifications, and formulation adjustments to minimize side effects and ensure safe and effective drug delivery [22] (Fig. 4).

The potential for off-target effects and systemic toxicity associated with nanoparticle exposure is another critical consideration in nanomedicine formulations. Interactions with biological components such as proteins, cells, and tissues may lead to unintended biological responses and adverse effects. Strategies such as surface modification, targeted ligands, and dosage optimization offer avenues to enhance specificity, minimize systemic exposure, and mitigate off-target effects, thereby optimizing therapeutic efficacy while reducing the risk of toxicity [21].

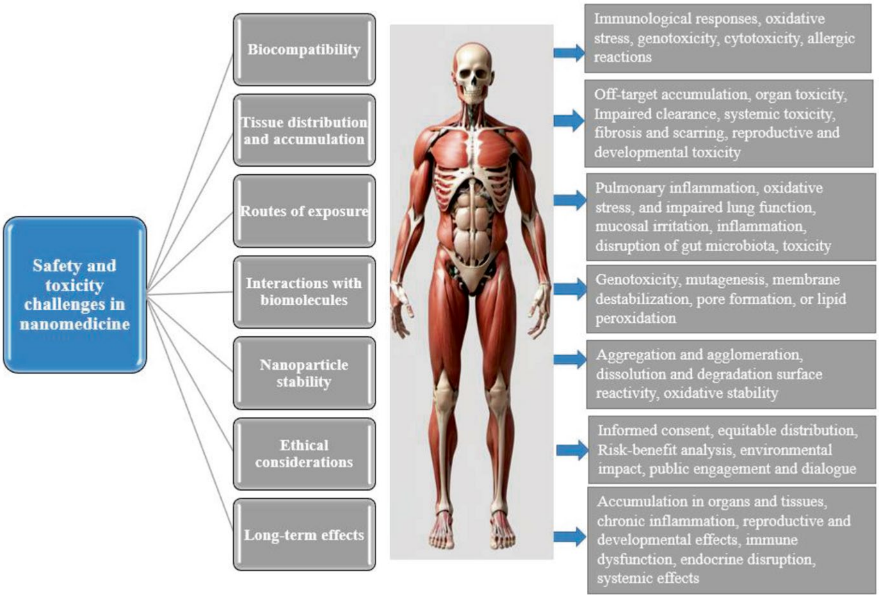

Safety and toxicity challenges in nanomedicine
Biocompatibility
Tissue distribution and accumulation
Routes of exposure
Interactions with biomolecules
Nanoparticle stability
Ethical considerations
Long-term effects
Immunological responses, oxidative stress, genotoxicity, cytotoxicity, allergic reactions
Off-target accumulation, organ toxicity, Impaired clearance, systemic toxicity, fibrosis and scarring, reproductive and developmental toxicity
Pulmonary inflammation, oxidative stress, and impaired lung function, mucosal irritation, inflammation, disruption of gut microbiota, toxicity
Genotoxicity, mutagenesis, membrane destabilization, pore formation, or lipid peroxidation
Aggregation and agglomeration, dissolution and degradation surface reactivity, oxidative stability
Informed consent, equitable distribution, Risk-benefit analysis, environmental impact, public engagement and dialogue
Accumulation in organs and tissues, chronic inflammation, reproductive and developmental effects, immune dysfunction, endocrine disruption, systemic effects

Fig. 4 Safety and toxicity challenges in nanomedicines

Additionally, navigating regulatory pathways and facilitating clinical translation pose significant challenges for nanomedicine formulations, necessitating comprehensive preclinical and clinical evaluations to validate safety, efficacy, and quality. Regulatory bodies like the FDA and EMA impose stringent requirements for preclinical safety assessments, manufacturing standards, and clinical trial protocols. Early identification and resolution of regulatory issues are crucial for obtaining timely approval for clinical use [22].

Furthermore, scalability and cost-effectiveness are pivotal considerations for large-scale production and commercialization of nanomedicine formulations [68]. Developing manufacturing techniques that are both scalable and cost-effective is essential for ensuring broad accessibility and utilization, particularly in resource-limited settings. Preserving product quality and consistency while minimizing production costs is essential for enhancing the widespread adoption of nanomedicine formulations [69].

6 Conclusion

The complexities surrounding safety and toxicity in nanomedicine necessitate a multidisciplinary approach that encompasses meticulous assessment, strategic formulation design, and proactive mitigation strategies. Despite the significant potential of nanomedicine to revolutionize drug delivery and improve therapeutic outcomes, its successful adoption in clinical settings hinges on effective management of safety concerns and minimization of toxicities. Innovative strategies aimed at enhancing the safety profile of nanomedicine formulations while optimizing therapeutic efficacy include rational design, surface modification, targeted delivery, and combination therapy. Moreover, comprehensive assessment methodologies such as physicochemical characterization, *in vitro* and *in vivo s*tudies, immunotoxicity evaluation, and long-term safety assessments play pivotal roles in identifying potential hazards and addressing safety concerns.

Adherence to rigorous quality standards and regulatory requirements is paramount in ensuring the safe and effective integration of nanomedicines into clinical practice. By adopting proactive and holistic approaches to safety and toxicity challenges, researchers can fully harness the potential of nanomedicine to address unmet medical needs and improve patient care. Ultimately, sustained collaboration, innovation, and dedication to ensuring patient safety and therapeutic efficacy are indispensable for the successful integration of nanomedicine into clinical practice.

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Nanomedicine's Role in the COVID-19 Vaccine Development and Optimization

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Abbreviations

APCs	Antigen Presenting Cells
BCR	B Cell Receptor
CpG ODN	Cytosine–guanine dinucleotide-containing oligodeoxynucleotide
DTaP	Diphtheria, Tetanus, Pertussis
HIV	Human Immunodeficiency Virus
MHC	Major Histocompatibility Complex
NSPs	Non-Structural Proteins
NTPase	Nucleotide Triphosphatase
ORF	Open Reading Frame
PAMPs	Pathogen Associated Molecular Patterns
RBD	Receptor Binding Domain
siRNA	Small Interfering RNA
TLR	Toll-like Receptor
UTR	Untranslated Region
WHO	World Health Organization

1 Introduction

In December 2019 world encountered a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19). Being an airborne pathogen & a respiratory RNA virus it spreads worldwide very rapidly. Globally SARS-CoV-2 caused more than seven hundred

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million cases and seven million deaths till March 2024 [1]. In December 2020 Emergency Utilization Authorization (EUA) of two m-RNA-based vaccines BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) from the US-FDA is in record time in the history of Vaccine biology. In addition to the m-RNA vaccine, nanomedicine plays a significant role in the development and delivery of live attenuated/inactivated virus vaccines, protein Subunit based vaccines, virus-vectored, Viral-like Particle (VLP) vaccines and DNA vaccines. The WHO data suggests that nearly 200 vaccine candidates are still in clinical phase [2]. After the discovery of mRNA in 1961 by Brenner et al, nearly two decades people were not able to study it further due to rapid mRNA degradation. In the 1980s scientists were able to transcribe the mRNA in vitro and cationic lipids were used for the very first time to encapsulate mRNA for injection and transcription into mouse quadriceps muscles [3]. Immunization of mice with influenza-virus nucleoprotein encapsulated in liposomes and anti-influenza Cytotoxic T-cell response laid the foundation of mRNA vaccines [4]. Apart from the stability, the safety of mRNA vaccines was the next big question for it's clinical use. Kariko et al in 2005 discovered that if mRNA is synthesized by modified uridine, it could escape from recognition and degradation by the immune system. Modified mRNA increased the stability and immunogenicity in vitro and heralded a new era of vaccine formulations [5]. Since the onset of COVID-19, mRNA vaccines have emerged as a focal point of research due to their short research and development timelines, straightforward production methods, and ability to trigger a robust immune response.

In certain cases, instead of the whole pathogen as live, attenuated, killed, or inactivated, only the pathogen-derived part or subunit is feasible or advisable to use for vaccine preparation. This strategy can employ antigenic proteins from pathogens, glycoproteins, and exotoxins produced by infectious agents. A subunit vaccine was introduced against *Bordetella pertussis* a causative agent of whooping cough in 1972 followed by a new version of DTaP in the 1990s. Novavax created NVX-CoV2373, the first subunit vaccine that received EUA against SARS-CoV-2 using RBD (receptor-binding domain of SARS-Cov-2 spike protein) cloned into baculovirus vector and expressed in vitro in insect cells. Matrix-M is an adjuvant derived from the *Quillaja saponaria* plant included as a component of NVX-CoV2373 and amphiphilic detergent polysorbate 80 (Tween 80) is used for self-assembly of micelles displaying pre-fusion stabilized spike proteins [6]. Several viral vectors based on the Vaccina virus, Adenovirus, vesicular stomatitis virus, and canarypox virus have been used in the past for vaccine formulation. Johnson & Johnson Covid 19 vaccine used human adenovirus 26 (Ad26) as a vector carrying information for spike protein expression. Ad26 was preferred as a viral vector because the majority of the population had not been exposed to this virus earlier thus avoiding the secondary response to the vector. On the same path, Astrazanecea's vaccine used Chimpanzee adenovirus (ChAdOx1) to avoid the immune memory risk [7]. Nanomedicine also helps in engineering self-assembled particle comprised of macromolecules i.e. viral proteins that mimics the native virus but lacks the genetic material. In this chapter, we are giving an overview of how nanomedicine is employed in the formulations of various types of vaccines against SARS-CoV-2, with an emphasis on the role of nanotechnology during this process.

2 Advantages of Nanotechnology for Vaccine Development

Nanomedicine is an interdisciplinary science that arose from the amalgamation of biomedicine, nanotechnology, and pharmaceutical sciences for development of novel nanoformulations having nanoparticle sizes ranging from 1 to 100 nm as defined by the Food and Drug Administration (FDA) for therapeutic purposes [8]. Nanomaterials are adopted widely as nanocarriers for drug delivery [9, 10]. In case of immune activation, Nanotechnology allows a nono-level perspective which helps in antigen delivery, and immunoboosting and mimics the natural interplay between viruses and the immune system [11]. Nanotechnology bridges the gap created due to lack of CD8+ immune response, a major defect of traditional vaccines because they rarely activate classical MHC-1 mediated presentation and cross-representation. Nanoparticle activates the cross-representation pathway by releasing antigen in cytoplasm by endosome escape (discussed later) mechanism in a pH-responsive manner [12]. Vaccine particles engulfed by migrated DCs at the injection site as well as also accumulated in draining lymph node and bind to the B cells in Germinal Centres (GCs) either directly or represented through resident APCs (Follicular Dendritic cells and or macrophages) in subcapsular sinus or medulla of Lymph node in MHC-II independent manner [13, 14]. Activated B cells then undergo proliferation, differentiation and somatic hyper mutation to secrete high affinity antibodies with the help of Tfh (T follicular helper cells) [15]. At this point nanomedicine played an important role in the improvisation and production of robust antibody response by three major approaches: (a) increasing the accumulation of antigens in the draining lymph node, (b) to display the antigens in a repetitive and organized way to BCRs that mimic the natural structure of a virus, (c) to improve the cooperation of Tfh cells with GC B cells [16]. Antigens conjugated with multiple nanoparticles exhibit higher lymph node accumulation as compared to free antigens [17, 18]. The lymph node accumulation is high for the particle size range from 20 to 200 nm on the other hand the large-size particle (500–2000 nm) are captured and retained at the injection site by Dendritic Cells (DCs) [19]. Phosphatidylserine incorporation in the liposomal bilayer enhanced the lymph node localization 3 times. The nanoparticle can be designed such that they could mimic the PAMPs structure for the optimization purpose of innate and humoral immune response. Many immune sensors i.e. TLR are multimer (pentamer or decamer) and can give better response after binding to repetitive antigens [20]. Repetitive antigen designing can promote BCR Crosslinking and clustering, a requirement for B cell activation. The dependency of T-helper cells for stimulation of B-Cell can also be reduced by designing heptenated polymer-like repetitive and organized antigen which are capable of activating B-cells without CD4+ T cells co-stimulatory signals.

Collectively, all of the above-mentioned tactics can enhance antigen uptake by APCs and diminish the threshold for B cell activation [21].

3 Nanoparticle-Based Vaccines

Nanomedicines have many advantages in the treatment of viral infections, including easy fabrication, tunable antiviral properties, flexible surface engineering, controllable pharmacokinetics [22], desirable biosafety [23], and compelling antiviral functions. In past, they are developed for various viruses for example Herpes simplex, HIV, Human papilloma virus, and respiratory viruses [24]. Based on antigen loading strategies nano-formulations can be divided into two major categories: nanoparticle encapsulating nucleic acid material and vaccine antigen within their core and nanoparticles that display vaccine antigen on their surface, antigen-presenting nanoparticles [25]. To combat COVID-19 and newly emerging variants of concern (VOC), various nanocarriers such as Lipid nanoparticle (LNP), Circular RNA Vaccines, Polymer vaccine nanoparticles, Protein nanoparticle (PNP) and or Viral like particles (VLPs), liposome membrane based nanoparticles are developed, and a number of them are still in the process even today.

3.1 Lipid Nanoparticle

In 2023, Katalin Kariko and Drew Weissman were awarded Nobel Prize in physiology and medicine for their research which laid the foundation for the development and delivery of lipid nanoparticle-based mRNA vaccines. In 2005, Kariko et al demonstrated that nucleoside modification of mRNA from uridine to pseudouridine could avoid its degradation from the innate immune mechanisms, as a result, the half-life and antigenicity of mRNA is increased [5]. The entry of mRNA inside the cell was challenging because of its negative charge, hydrophilic nature, large size whichdoes not allow naked mRNA to cross the hydrophobic lipid bilayer of cell membrane. To solve this problem various delivery vehicles developed to encapsulate the active substance (i.e mRNA, DNA, siRNA) like Lipid nanoparticle (LNP), Polymeric Nanoparticle, Lipopolyplexes (LPP) and cationic polypeptide. Lipids and their derivatives are favored over other nanomaterials due their biological compatibility, higher encapsulation rate and low immunogenicity. The initial description of liposomes, the traditional form of lipid nanoparticles, dates back to 1965 [26]. Liposomes are spherical vesicles made of one or more concentric phospholipid bilayers surrounding an aqueous core having a size of 20–1000 nm. They self-assemble from phospholipids or synthetic amphiphiles, often aided by cholesterol to enhance stability through tight packing [27]. Liposomes represent one of the pioneering nanomedicine delivery platforms to successfully transition from conceptualization to clinical application due to their structure which allows them to carry both hydrophilic and hydrophobic drugs, making them suitable for a wide range of therapeutic applications.

Even though liposomes have been studied for three decades as a promising vehicle for various drug molecules, it was in 1993 that Crucell Berna Biotech of Switzerland pioneered the development of the first liposome-based vaccine, Expaxal, targeting hepatitis A [28]. The succeeding iteration of LNPs, encompassing solid LNPs, nanostructured lipid carriers, and cationic lipid-nucleic acid complexes, exhibit heightened complexity in internal composition, improved stability, and enhanced targeting capabilities [29]. Lipid nanoparticles paved the path for the development of almost all present-day COVID-19 mRNA vaccines. Lipid nanoparticles consist of four lipid components: ionizable lipids, helper phospholipids, cholesterol, and PEGylated lipids (Fig. 1a), although the structural designs of COVID-19 mRNA vaccines differ significantly among various developers.

(a) Ionizable lipids LNPs were designed initially to be used as a new drug delivery platform for cancer immunotherapy [30]. Currently, several proprietary ionizable lipids are extensively utilized for nucleic acid delivery in various mRNA-based COVID-19 vaccines. Two leading COVID-19 vaccines, mRNA-1273

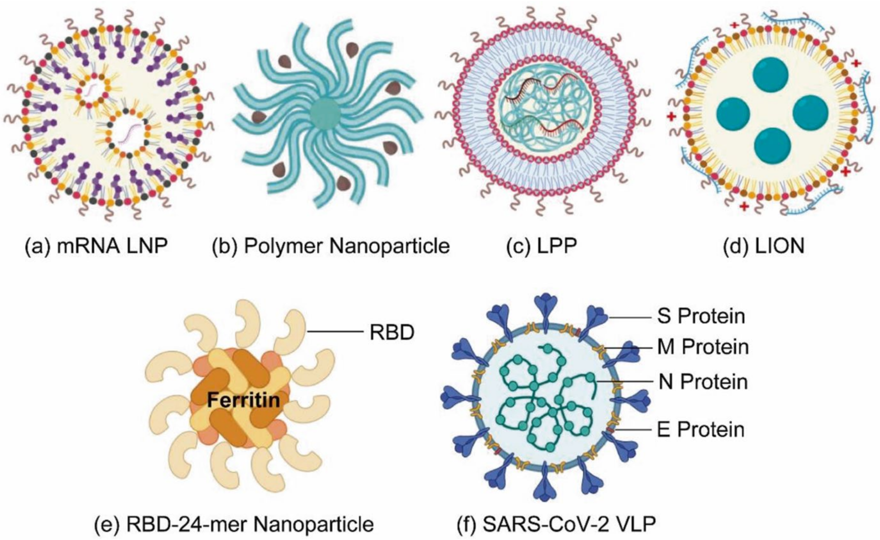

Fig. 1 Nanoparticle platforms utilized in the designing of COVID-19 vaccines. m-RNA based Nanoparticle vaccines (**a**–**d**) consist of mRNA encoding full-length Spike protein, helper lipids, ionizable cationic lipids, and cholesterol in Lipid nanoparticle (LNP) (**a**); Lipopolyplex (LPP) nanoparticles have polymer encapsulated mRNA (**b**) in the core and outer lipid shell composed of PEGylated and Ionizable lipid (**c**); self-amplifying RNA (saRNA) are complexed with cationic lipids by charged interaction having squalene and SPIO in core known as Lipid InOrganic Nanoparticle (LION) (**d**). Ferritin protein conjugated RBD-24-mer nanoparticle vaccine represents Protein nanoparticle (PNP) vaccines (**e**). Viral-like nanoparticles (VLPs) expressed all structural proteins i.e. M, N, E, and Hexapro S antigens of SARS-CoV-2 (**f**). (Figures are created with BioRender.com)

developed by Moderna and BNT162b2 by Pfizer/BioNTech, employ cationic lipid nanoparticles (LNPs) to transport the mRNA encoding the complete SARS-CoV-2 spike protein. The Spike mRNA is undergone substitution of two proline (2-P) and deletion of furan cleavage site to express stable prefusion conformation of S protein and also contribute in improving immunogenicity [31]. The negatively charged RNA binds to positively charged ionizable lipids in acidic environment. The ionizable lipids utilized possess a pKa below 7.0, rendering them neutral under physiological conditions. This characteristic enables their transport through interstitial and lymphatic fluids and makes them a safer and more stable option as a delivery method for vaccines. Upon entry into acidified endosome compartments with a pH below 6.0, these lipids undergo conversion to a positively charged state. This transformation is followed by lipid exchange and fusion with the negatively charged endosome membrane, facilitating the release of mRNA into the cytoplasm, where it undergoes translation into antigen proteins. This phenomenon, known as "endosome escape," is crucial for the process. Cationic lipids activate endosomal proton pumps and may aid in the release of nucleic acids into the cytoplasm [32]. Moderna developed SM-102 (9-Heptadecanyl 8-{(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino}octanoate), a larger branch ionizable lipid with ester bond for easy biodegradability to use in mRNA-1273. This lipidis chemically similar to ALC-0315 ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) used by BioNTech (Table 1), [33–35].

(b) Helper lipids being amphiphilic lipids, play a crucial role in maintaining the lipid bilayer structure, facilitating fusion with endosomal membranes, and determining the specificity of target organs. A saturated lipid DSPC (1,2-distea royl-sn-glycero-3-phosphocholine) is the only FDA approved phospholipid molecule used in both Covid-19 mRNA vaccines of BioNTech and Moderna [36]. Together with the electrostatic interaction of the cationic head with mRNA, ionizable lipids formed an electron-dense core in the presence of DPSC and Cholesterol.

(c) Cholesterol, a natural lipid, can influence the bilayer structure of biological membranes in diverse ways, such as adjusting fluidity, thickness, compressibility, water penetration capacity, and intrinsic curvature [37]. Within lipid nanoparticle (LNP) formulations, cholesterol enhances stability by filling gaps among LNP molecules and facilitating fusion with endosomal membranes. Consequently, this process promotes the uptake of the vaccine complex [38].

(d) Lipids with covalently attached polyethylene glycol (PEGylated lipids) are the least abundant component of LNPs. The mRNA containing electron-dense core of LNPs are surrounded by monolayer composed of PEGylated lipids, cholesterol and helper lipids. Hydrophilic PEG is linked with hydrophobic anchoring lipid dimyristoyl glycerol (DMG). Both substances impact the size, permeability, and immunogenicity characteristics of LNPs [39]. The primary function of PEGylated lipid within LNPs is to mitigate aggregation and nonspecific uptake by immune cells, nonspecific adsorption of serum proteins and increase colloidal stability. PEGylated lipids also affects transportation efficiency,

Table 1 Nanoparticles-based representative vaccines used against SARS-CoV-2 and its variants

Nanomedicine	Components	Mechanism	Organism	Variant targets
BNTI162b2	Ionizable cationic lipid: ALC-0315 Helper lipids: DSPC, Cholesterol, PEG-DMA mRNA encoding the full-length Spike protein of wild-type strain	BNT162b2 and mRNA-1273 vaccines encoded the S protein antigen from the wild-type strain, and a third booster dose effectively triggered a strong immune response by activating cytotoxic T lymphocytes and generating neutralizing antibodies.	Human	Alpha (B.1.1.7), Beta (B.1.351), Delta (B.1.617.2), omicron (B.1.1.529)
mRNA-1273	Ionizable cationic lipid: SM-102 Helper lipids: DSPC, Cholesterol, PEG-DMG mRNAs encoding the full-length Spike protein of wild-type strain		Human	Alpha (B.1.1.7), Beta (B.1.351), Delta (B.1.617.2), omicron (B.1.1.529)
mRNA-1273.351	Ionizable cationic lipid: SM-102 Helper lipids: DSPC, Cholesterol, PEG-DMG Variant-modified mRNAs encoding the Spike protein of the Beta variant	mRNA-1273.351 encoded the S protein antigen of the Beta strain, and a third booster resulted in higher neutralizing antibody levels against the Beta variant.	Human	Beta (B.1.351)
SW0123	Cationic polymer: SW-01 Ionized and non-ionized lipids, Ionizable lipid, DOPE, PEG-lipid mRNA encoding the full-length S protein of wild-type strain	SW0123 elicited strong IFN-γ+CD8+ T cell reactions, Th1-type CD4+ T cell reactions, and elevated levels of antibodies specific to RBD	Mice	Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1)
Ferritin-RBD nanovaccine	A 24-mer RBD sortase-A-conjugated ferritin nanoparticle Aluminium and 3M-052 adjuvants	The nanovaccine induced powerful neutralizing antibodies that effectively neutralized coronaviruses originating from various origins.	Mice, macaques	Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1)
S-6p VLPs	VLPs expressing all structural protein antigens (M, N, E and HexaPro S) Aluminium and K3-CpG ODN adjuvants	The multi-valent SARS-CoV-2 VLP induced elevated levels of IgG antibodies against S, RBD, and N antigens, prompting versatile Th1-skewed immune reactions, diminishing viral burdens, and averting lung impairment	Human	Alpha (B.1.1.7)

the anchoring lipids generally have 10–18 carbon, and the greater the tail length of the anchoring lipid, the longer the half-life of the LNP complex in the host [40]. PEG2000-DMG and ALC-0159 (mPEG-DTA) are PEGylated lipids used in Moderna and BioNTech Covid-19 mRNA vaccines, respectively [41, 42].

3.2 Polymer/Lipopolyplex (LPP) Based Vaccines

All FDA-approved LNP-based vaccines fall under non-amplifying (replication-deficient) mRNA vaccines. Other than cationic lipids in LNPs, polymers are another broadly used mRNA delivery system. Use of polyesters in carrying mRNA addressed the degradability issue of polymers i.e. lipid encapsulation of poly(beta-amino esters) (PBAE) with mRNA loaded on the surface of LNPs through electrostatic interactions (Fig. 1b) [43]. Lipid-shell coated Lipopolyplex (LPP) comprising of compact mRNA core enveloped in a lipid shell. LPP found to be more stable, less cytotoxic and have higher endosomal escape efficiency. Similar type of LPP technology was employed to generate COVID-19 mRNA vaccine SW0123 by encapsulating the PBAE—mRNA complex in a lipid shell comprised of a ionizable lipid, DOPE (1,2dioleoyl-sn-glycero-3-ethylphosphocholine), PEgylated lipid have shown promising results in non human primates and mice (Fig. 1c). So, the only difference between LNP and LPP lies in dense core, where a cationic polymer is in complexi electrostatically with mRNA encoding full length S protein instead of lipid-mRNA complex in LNPs [44]. Blakney et al developed cationic polyamidoamine polymer (named pABOL) that is used for delivery of saRNA (self-amplifying mRNA, discussed below) of spike protein for vaccine formulation found to be less immunogenic as compared to LNP-formulated saRNA despite the higher level of transgene expression [45, 46].

3.3 saRNA-Based Vaccines (NLC, Protamine-Based Delivery)

Self-amplifying RNA (saRNA) vaccines are derived from genomes of RNA viruses *Alphavirus* or Flaviviruses. Alphaviruses are engineered to express and amplify the inserted target antigens using their replication machinery. saRNA vaccines contain genes responsible for encoding the target antigen, along with the viral replicase enzyme machinery, enabling their self-amplification. Around 12 kb ORF post-5′ UTR encodes four Non-structural Proteins (nsPs 1, 2, 3, 4) of alphavirus. These nsPs serve various functions such as mRNA capping, NTPase/helicase/protease activity, macrodomain function, or RNA-dependent RNA polymerase function. After the translation of saRNA by endogenous ribosomal machinery, the nsP precursor assembles the early replication complex and positive strand synthesizes a replication intermediate negative-strand RNA. Cleavage of nsP precursors yields a late replication complex. Subsequently, the negative-strand RNA from the replication intermediate is used to synthesize full-length positive-strand genomic RNA and a subgenomic positive-strand RNA encoding the antigen. Thus, a single saRNA molecule initiates self-amplification of antigen genes within the cell by generating multiple RNA transcripts. saRNA vaccines are required in ultra-low doses (0.1–10 µg) as compared to conventional non-replicative moderna and BioNTech vaccine (30–100 µg) for generating similar immune responses. saRNA vaccines are stable at higher temperature, non-integrative and safe, level of antigen expression is quite high, induce type-I interferon response and because of its low dose it creates a scope for delivery of some other vaccine in combination. Numerous saRNA vaccines tested for COVID-19 are in preclinical settings and a few of them are in 2 and 3 phase of clinical trials [47-49]. The requirement of large size delivery vector which accommodate alteast 9–13 kb sequence and encapsulation efficiency undermines the relevance of saRNA vaccines. To circumvent this problem, bipartite split replicons in which one encodes nsP of alphaviruses and another expressing gene of interest are developed, while still consverving self-amplification qualities of replicon RNA [50]. In addition to LNPs other lipid based delivery methods has been discovered for encapsulation of saRNA vaccines. Among several Alphavirus vectors, Venezuelan equine encephalitis virus (VEEV) vectors derived replicons were used extensively in numerous preclinical studies. After keeping VEEV in centre stage Erasmus et al develop Lipid InOrganic Nanoparticles (LION) delivery vehicle to express full length spike protein of VOCs (variant of Concerns) of SARS-CoV-2. LION is a cationic emulsion consists of hydrophobic squalene (having adjuvant effect also) core with inorganic superparamagnetic iron oxide (Fe_3O_4)/SPIO to enhance stability, nonionic surfactants, the hydrophobic sorbitan ester (Span) span 60, the hydrophilic ethoxylated sorbitan ester (Tween) tween 80 on the surface to maintain emulsive nature, and 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), a cationic lipid for electrostatic association with saRNA encoding spike on nanoparticle's surface (Fig. 1d). The independent manufacturing of LION facilitates the stocking of a vaccine which was later on found to be stable for up to 10 weeks at room temperature after premixing with separately produced repRNA. A single dose of LION-saRNA vaccine based on VEEV induced a potent SARS-CoV-2 neutralizing antibody (NAbs) and type-1 T helper cell reaction in nonhuman primates, alongside a moderate S-specific T cell response [51]. LION technology has a significant advantage over FDA-approved LNPs based mRNA vaccines in being dispensible to deep freezing, making it a popular choice in developing countries where cold chain maintenance is not possible. This vaccine is under phase 1 clinical trial in India by name HDT-301. A similar formula adopted in non -structured lipid Carrier (NLC) based LNPs in which SPIO is replaced by a solid lipid composed of saturated Triglyceride and rest of the components are similar to LION. The potential for employing NLC/saRNA for SARS-CoV-2 vaccination was assessed by examining a VEEV-derived replicon encoding the S protein [52]. In addition to Lipids and polymers, peptides can also be used for mRNA delivery. The property of amphiphilic or cationic amino groups carrying amino acids has been exploited in generating peptide carriers that can establish charged interactions with mRNA to form nano complexes. Liposome-protamine-RNA (LPR) nanoparticle prepared by condensation of saRNA with protamine through electrostatic interactions were used to deliver VEEV-based RBD encoding replicon in mice are shown to target draining lymph nodes and eliciting Innate immune response by TLR signaling [53].

3.4 Circular RNA (CiRNA) Vaccines

CirRNA due to end closed structure is comparatively less susceptible to degradation through exonucleases than linear mRNA. CirRNA usually devoid of essential elements for cap dependent translation, was identified earlier in yeast mitochondria and hepatitis D virus. Recently Liang et al developed a RBD antigen encoding CirRNA, encapsulated in which was fused with signal peptide of tissue plasminogen activator (tPA) for ensuring secretion of antigen. Further, bacteriophage T4 fibritin was added to C-terminus for trimeric receptor conformation. For translation of gene Internal ribosome entry site (IRES) was inserted in front of antigen coding sequence, so that circular RNA could be produced using group 1 ribozyme autocatalysis reaction. After immunizing mice with two different intramuscular doses of candidate vaccine long-lasting neutralizing antibodies and Th1-biased cellular immune responses were observed [54].

mRNA vaccines offer several advantages over whole-virus, subunit proteins, and DNA vaccines. Firstly, they boast a superior safety profile due to their non-infectious nature and lack of potential genomic integration risks, reducing tumorigenesis concerns. Additionally, mRNA is easily biodegraded, with controllable half-lives through chemical modification, enhancing safety. Moreover, mRNA vaccine development is faster and less resource-intensive compared to whole-virus vaccines, bypassing the need for virus culturing and featuring highly yielding in vitro transcription reactions. Lastly, mRNA's ability to trigger innate immune responses via TLR7/8 and TLR3 confers intrinsic adjuvanticity, which can be further enhanced through modifications.

4 Viral-Like Particles (VLPs)/Protein Nanoparticle (PNPs)

The self assembled protein nanoparticles (SANPs) can be categorized in to two groups; one those having conjugated virus-derived peptides or subunit for delivery purpose and in second, the strategy involves assembling the subunits themselves into antigen nanoparticles to enhance immunogenicity. VLPs are the extension of subunit or epitope-based vaccines. By looking at size and structure VLPs seem like "empty shells mimics of the enveloped viruses" but lack genetic material with a size of 15–30 nm [55]. Various production system options like Yeast, prokaryotic cells, insects, plants and mammalian cells can be explored for VLPs [56]. In addition to the advantages of nanoparticle based vaccines over traditional vaccines discussed earlier, the VLPs and PNPs both presents antigens in its original form, which is destructured some times in case of inactivated vaccines. There are almost 5–6 VLPs vaccines reached up to clinical trials. HexaPro-S is a six proline substituted prefusion-S protein alum adsorbed vaccine created by transient cotransfection HEK293T cells with four SARS-CoV-2 structural proteins (S,M,N,E) encoding gene constructs (Fig. 1f). It was reported that six proline substitution increases the protein yield significantly and Prefussion-S protein is found to be stable in freeze-thaw cycling and as well as in heat stress. In K18-ACE-2 transgenic mice, a single dose of subcutaneously injected HexaPro-S with CpG ODN adjuvant generated a robust level of anti-S, anti-RBD, and anti-N IgG antibodies, leading to multifaceted Th1-biased immune reactions, diminished viral presence, and safeguarded lung health following exposure to live virus challenge (Table 1), [57]. In addition to producing virus-like particles (VLPs), there has been extensive exploration into using non-viral protein-based nanoparticles (NPs) or protein-based biomaterial scaffolds as vaccine carriers. Ferritin has recently become a notable platform for displaying antigens. Alongside its ability to self-assemble, the ferritin protein complex demonstrates impressive resistance to thermal and pH fluctuations. Additionally, it exhibits characteristics such as monodispersity, uniform small size, biocompatibility, biodegradability, cost-effectiveness in large-scale production, a hollow cavity enabling reversible assembly/disassembly, and compatibility with surface conjugation through chemical or genetic methods. The 24-meric ferritin nanoparticle displaying 24 copies of RBD of SARS-CoV-2 elicit significant immunogenicity for both wild type viruses and pseudoviruses (Fig. 1e). I53-20 a 120 mer nanoparticle exhibiting 60 copies of RBD induced 10 times more antibody than full-length Spike protein of SARS-CoV-2 as well as protecting Non-human primates from infection. 60-meric lumazine synthase nanoparticle induced long lasting neutralising antibodies against the VOCs (B.1.351, B.1.1.7 and P.1) and SARS-related bat coronaviruses [58]. SpyCatcher-mi3 another 60-mer vaccine displaying RBD is temperature resistant and used after lyophilization showed protective effect in pigs and mice [59]. A quadrivalent mosaic nanoparticle vaccine, showing S proteins from the wild type and three variants B.1.1.7, B.1.351 and P.1, generated higher levels of neutralizing antibodies against the mentioned one but also the extended crossprotectivity for B.1.617.2 and B.1.525 in both mouse and non-human primate models [60]. One of the reasons that none of these PNPs could get FDA approval for EUA is the heterologous-produced proteins which lacks glycosylation at important sites that aids in the stability and potent immunogenic response.

5 Cell Membrane Derived Vesicles and DNA Vaccine Delivery

Cell membrane based nanocarriers comes in to picture when the antigen of interest has optimum immunogenicity and biocompatibility only with the original cell membrane with natural composition. In recent years Exosome Vesicles (EVs) are explored in context of cell membrane-derived nanoparticles those having highly stable delivery system with better targeting capabilities. In comparison to other LNPs, EVs preserve the original conformation of antigen, less immunogenic, less toxic and have abilities to cross biological barriers, making them favorable choice for vaccine development. The EVs can be enginnered for the surface expression of different antigens simultaneously i.e. a chimeric Spike protein is designed such away that its transmembrane structure replace the G protein of Vesicular Stomatitis virus and as a result of which loading of S protein on EV is increased. Two distinct SARS-CoV-2 vaccines employing an EV-based method: one involves the utilization of vectors containing the four structural proteins (S, N, M, and E) of SARS-CoV-2, which are introduced into HEK293 cells, resulting in the release of EVs (or VLPs) carrying the complete spectrum of viral antigens in their native configuration. This nano-vector vaccine, capable of transporting multiple proteins, has the potential to trigger a more potent immune response in the host organism [61]. Nanodecoy represents another type of nanoparticle derived from cell membranes. Through the fusion of genetically engineered cell membrane-derived nanovesicles that express virus surface antigen receptors, nanodecoys can efficiently absorb viruses. ACE2-based nanodecoys could interact with the S protein of SARS-CoV-2 and variants to inhibit viral entry, thereby protecting the host cells from infection [62]. Moreover, due to their natural capacity to trap pathogens, nano decoy-pathogen complexes could also be leveraged in vaccine development because in the process of thermal or chemical inactivation of a virus can lead to alteration in viral antigen and its immunogenicity.

Nanomedicine was also deployed for the needle-free delivery of DNA vaccines. Proteoplipid Vesicles (PLVs) made up of neutral lipid layers in which a fusion-associated transmembrane protein incorporated is developed. The fusion-associated small transmembrane protein is originally secreted by fusogenic reoviruses, but in PLVs its job is to induce lipid exchange and membrane fusion followed by syncytium formation. Based on this formulation, PLVs can efficiently merge with the plasma membrane of target cells. This allows them to deliver the loaded vaccine directly into the cytoplasm, circumventing the endocytic pathway and enhancing the vaccine's entry efficiency into the cytoplasm [63].

6 Optimization of COVID-19 Vaccines for New Variants and Potential Future Pandemics

The emergence of novel immunogenic viruses over the genomic framework of existed or eradicated viral species is the expected trajectory of a pandemic due to sustained diversification and adaptation cycle. Coronaviruses have very broad host range across birds and mammals. Bidirectional transmission of virus from humans to animals and after a period of adaptation again Zoonotic transmission back in to humans can be the root cause of future reemergence of new variants or novel coronavirus with additional evasion strategies. The existing nanomedicine platforms need to be optimized or tuned to stimulate a robust long lasting nAbs and Tcell response against future variants and novel strains. Nanotechnology answered limitations of non-particulate antiviral therapy such as stability and bioavailability by modifying pharmacokinetics, toxicity, and effective dose concentration reduction in circulation but the challenge of subtherapeutic concentration of antiviral drug to specific organs can be resolved by targeted organ-specific nanoformulations. During multiantigenic vaccine formulation addition of regions of virus that are conserved like Nonstructural proteins N, M or ORF1ab in addition to Spike, could potentially increase the protection against Variant of Concerns (VOCs) [64]. Computational analysis to identify the immunodominant epitopes of Spike protein which can elicit T and B-cells responses can be another approach for augmenting both humoral and cell-mediated response. Another benefit of epitope-based vaccines is that there is smaller polypeptide expression compared to the entire S protein. This size reduction could enhance expression levels, potentially enabling the utilization of lower doses of nucleic acids [65]. Before vaccination roll-out expansion of SARS-CoV-2 specific T cells are detected in blood even before PCR positivity and in mild and asymptomatic cases. It suggests that T cells are important in early viral control and infection blockade [66]. Nanoparticle vaccines capable of inducing robust resident T-cell memory responses could offer a means to achieve long-lasting protection against both existing and forthcoming variants of concern (VOCs). Alternative routes of vaccination like intranasal, sublingual, and aerosol administration need to be explored for inducing localized mucosal immunity at the site of infection. Notably, the introduction of RBD on protein nanoparticles triggered stronger cytotoxic CD8+ T cell reactions in mice compared to when the antigen was presented in its monomeric form [67]. Also in this line, the co-display of heterologous vaccine antigens i.e. RBD regions from human and animal coronaviruses may promote the recruitment of cross-reactive B cells in the immune response triggered by the vaccine. This, in turn, could expand the spectrum of variants or pathogens targeted by neutralizing antibodies. Mosaic nanoparticles generated by SpyTag/SpyCatcher 003-mi3 platform displaying 4–8 distinct RBDs from Zoonotic betacoronavirus and SARS-CoV-2 are evaluated for crossreactive nAb response [68]. Reverse vaccinology is a promising computational approach for molecular analysis of comparative similarity between viral antigen and host proteins. Molecular mimicry of human proteins is one of the classical examples of immune evasion by viruses [69, 70] and the inclusion of unique candidate peptides during antigen design could lead to the blockade of evasion. On the contrary, excluding similar sequences may reduce the generation of self-reactive autoantibodies [71, 72]. The availability of various nanoparticle vaccines could facilitate the implementation of heterologous prime-boost approaches. These strategies have the potential to reduce the effects of delays in the supply chain and offer more flexibility in vaccination programs. The combination of two or more established vaccine platforms can yield highly effective immune responses. In mice, administering adenovirus ChAdOx1 alongside a saRNA-LNP vaccine led to heightened antibody levels and an enhanced cellular immune response compared to using solely one type of vaccine [73]. Similarly, in rhesus macaques, a regimen involving a ChAd prime followed by a saRNA-LNP boost encoding prefusion stabilized S-protein resulted in robust T-cell and neutralizing antibody responses. To combat emerging viral variants the maximization of immunity is critical for vaccine mediated protection [74]. Boosting population immunity is key for maximizing vaccine protection and controlling emerging viral variants. In other noteworthy approaches for enhancement of immune response is to combined vaccines with immunostimulatory molecule i.e. 4-1BB, CD40, OX40 and blockade of immune checkpoint such as PD1, PDL1 and CTLA1. The OX40 agonist enhanced specific CD4+ T cell responses elicited by both protein and saRNA vaccines [75]. Viruses often engage with cellular glycocalyx components before binding to receptors. Polymeric nano disinfectants mimicking glycans, the key targets for viral attachment, to hinder this initial virus-cell interaction could be the option for desining next generation nanoparticles [76]. Recently, nanodisinfectants based on Titanium dioxide (TiO2), which possess inherent peroxidase-like capabilities, have demonstrated the ability to directly deactivate SARS-CoV-2 and its variants through the generation of reactive oxygen species [77].

7 Conclusion

For decades, researchers have been harnessing the advantages of nanoparticles for various purposes like the development and delivery of live attenuated/inactivated virus vaccines, protein Subunit vaccines, virus-vectored, Viral-like Particle (VLP) vaccines, DNA vaccines, and also in engineering self-assembled particle like viral proteins. The recent advancement came up with development of COVID-19 vaccines like vaccines BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) where unprecedented jump was observed in the global collaborative efforts. To combat COVID-19 and the newly emerging Variant of Concerns (VOC), a number of nanoparticles are still in the process even today. The ever-expanding spectrum of host range and reemergence forces to bring upon consistent effort in optimizing and fine-tuning against Variant of Concerns (VOCs). The extensive use of new nanoparticle vaccines has sparked safety worries due to rare yet severe reactions such as anaphylaxis and myocarditis post mRNA-LNP vaccinations. The presence of polyethylene glycol (PEG) 2000 in mRNA-LNP vaccines is thought to trigger anaphylactic responses. Urgent research is required to grasp the exact mechanisms and predisposing factors for these reactions, encompassing materials, and carriers beyond PEG, to reduce vaccine hesitancy and boost public acceptance of new vaccine technologies. Nanomedicine is a powerful and broad spectrum tool in biology and its applicability is seemingly limitless however a cautious risk assessment, regulations and balance between industry and academia is essential to facilitate the preclinical development of these innovative vaccines.

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Nanomedicine's Transformative Role in Advancing Cancer Treatment

Jai R. Vengurlekar, Bharti B. Bhaware, Tanishq Prasad, and Joyita Sarkar

1 Introduction

From the twentieth century, attempts have been made to cure cancer, an uncontrolled cell division and invasive medical condition to be treated with the help of nano-sized medications. Traditional cancer treatment methods like surgery, chemical-based therapy (chemotherapy), radiation based therapy (radiotherapy), etc. are not sufficient for the complete and healthy recovery of the affected individuals. They bring alongside effects like neuropathies, bone marrow suppression and gastrointestinal issues. Precision-based therapies like targeting and immunotherapy are viable options but challenges like drug resistance, relapse and auto-immune responses are faced.

With the help of nanotechnology, developments in the cancer therapy field are proving to be excellent alternatives to the challenges posed by the traditional methods [1]. Nano, term as defined by the International Union of Pure and Applied Chemistry are particles with 1–100 nm in dimensions, is sub-categorized as 0D, 1D, 2D and 3D (Table 1). These particles possess unique properties like compact size and high surface to volume ratio are key aspects to their applications in multiple fields. Their compact size helps them to travel through the bloodstream, across the cell membranes easily and also pass through the blood-brain barrier. They are easily retained in the system showcasing the enhanced permeability and retention (EPR) effect. With additional modifications to the surface, the clearance of the particles by immune system can be further reduced [2]. They also offer advantages like enhanced

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Table 1 List of nanoparticles based on dimensions

Dimension	Size(nm) and shape	Example
0-Dimensional [208–211]	Less than 100 in diameter	Quantum dots Magnetic nanoparticles Metal nanoparticles Up-conversion nanoparticles Polymer dots
1-Dimensional [212]	1–100 in length	Nanorods Nanotubes Nanofilaments Nanofibers Nanowires
2-Dimensional [213]	~5–10 in thickness	Metal nanosheets Graphene-based materials Transition metal oxides Dichalcogenides
3-Dimensional [214]	>100	Nano-cubes Fullerenes Dendrimers Nanocage

solubility and stability, controlled release and site-specific delivery with high bioavailability. To improve the systemic circulation and site-specific delivery, research is being conducted in various forms of drug delivery systems and particles like dendrimers, liposomes, polymeric micelles, and metallic nanoparticles, etc. (Fig. 1a). These carriers are loaded with the anticancer drugs and are targeted to their site of actions. Apart from drug delivery, nanoparticles are also used for imaging, adjuvant, etc. (Fig. 1b). Natural polymer like starch based nanoparticles are cost-effective and eco-friendly drug delivery systems having high drug loading capacity, biodegradability, and bio-compatibility. Likewise, metallic nanoparticles like gold, silver, platinum, copper, iron oxides, titanium, etc. are being researched for their electrical and catalytic properties that can be used for drug delivery and thermal imaging of the cancer cells. Along with these, metal-organic frameworks possessing porous structure and large surface area offer a well-defined structure, high porosity, and ease of functionalization [3].

Despite substantial advancements, there are still hurdles in getting nanoparticle-based cancer therapies from lab to bedside. Scalability of production, standardization of characterisation methodologies, and long-term safety profiles are all issues that must be solved for widespread clinical usage. This comprehensive book chapter will provide a complete examination of the present landscape of advanced nanoparticle-based cancer therapies. This work aims to inform researchers, clinicians, and policy makers involved in the development and application of innovative nanoparticle-based cancer treatment strategies by synthesizing recent advances, elucidating mechanisms of action, evaluating effectiveness across various cancer types, and discussing challenges and future directions (Table 2).

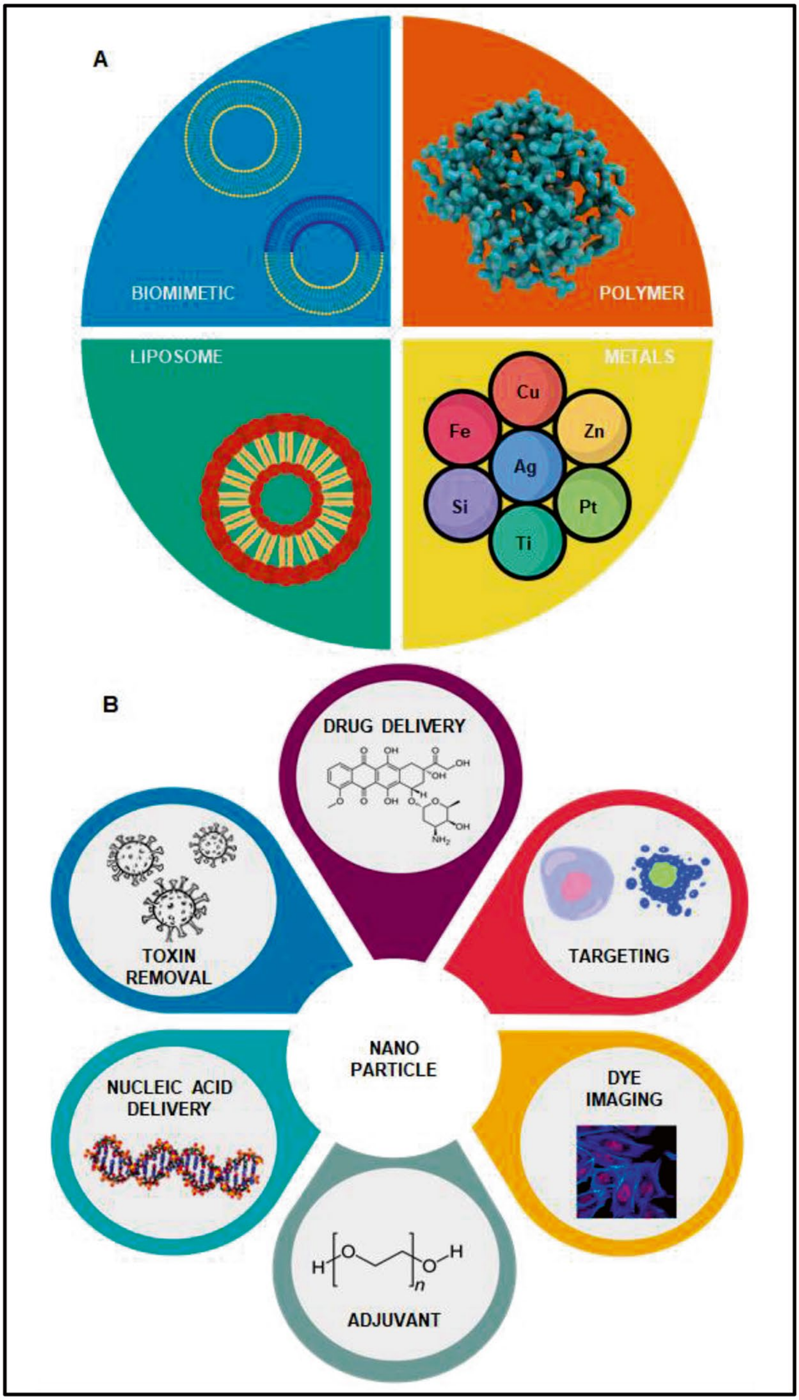
A
BIOMIMETIC
POLYMER
LIPOSOME
METALS
Cu
Fe
Zn
Ag
Si
Pt
Ti
B
DRUG DELIVERY
TARGETING
TOXIN REMOVAL
NANO PARTICLE
NUCLEIC ACID DELIVERY
DYE IMAGING
ADJUVANT

Fig. 1 Nanomaterials (**a**) Types (**b**) Applications

Table 2 Types of nano particles with their characteristics and applications

Nanoparticles	Size(nm)	Characteristics	Applications
Nanocrystals Quantum dots [215]	2–9.5	Bright fluorescence Narrow emission Broad UV excitation robust photostability	HeR2 surface labeling of cancer cells DNA hybridization, immunoassay Receptor-mediated endocytosis Long-term multiple-color imaging of liver cells
Carbon nanotubes [221]	0.5–3 diameter and 20–1000 length	Conducting Semi-conducting Insulating	The functionalization process improved solubility and the ability to enter the nucleus and cytoplasm of cells and use it as a carrier for transporting peptides and genes.
Dendrimer [216]	<10	Created by controlled polymerization, it is a highly branched, virtually monodisperse polymer system with three primary components: surface, branch, and core.	Extended circulation Regulated bioactive delivery Specific bioactive delivery to macrophages Liver targeting
Polymeric micelles [26, 217]	10–100 nm	Block amphiphilic copolymer micelles High payload Biostability Drug entrapment.	Target-specific long circulation Active and passive medication administration Diagnostic utility
Liposome [218]	50–100	Biocompatible Adaptable High entrapment effectiveness Phospholipid vesicles Simple to use	Long-term circulation provides passive and active transport of peptides, proteins, and genes.
Metallic nanoparticles [219]	<100	Minuscule size Large surface area Stable Suitable for functionalization.	Delivery of drugs and genes Susceptible diagnostic tests Thermal ablation Improvement of radiotherapy
Polymeric nanoparticles [220]	10–1000	Biocompatible Biodegradable Total drug protection.	Excellent payload delivery vehicle for steady and regulated dosing. Bioactive materials can be delivered actively and passively using surface-modified and stealth nanoparticles.

2 Mechanisms of Targeting of Nanoparticles

An essential factor for selecting a nanomedicine formulation for cancer therapy is its ability to precisely target cancer tissue while causing minimal adverse effects to normal tissue. The numerous nano-formulations utilized to deliver anticancer medications to tumour locations employ various targeting strategies [4–6]. The ability to precisely control temporal and spatial distribution of a drug is the primary advantage of nanoparticle based delivery systems over macro-systems. It can be accomplished by either passive or active targeting. While active targeting necessitates conjugation with antibodies, peptides, aptamers and small molecules, the passive targeting takes advantage of the EPR effect.

These targeting techniques accurately deliver the therapeutic payloads and diagnostics to affected areas and reduce the nanoparticle build up in the normal tissues. Similar underlying mechanisms are used by both therapies to build up nanoparticles inside malignant cells. Both targeted approaches in cancer nanomedicine utilise the pathophysiological characteristics of a tumour to build up nanoparticles. In order to enhance delivery to tumor, the researchers also employ design principles for nanoparticles that have been discovered empirically.

2.1 Passive Targeting

The phrase "passive targeting" is often used in nanomedicine to describe the deposition of nanoparticles in solid tumors. In 1986, Matsumura and Maeda made two major findings after administering nanoscale materials intravenously, laying the framework for passive nanoparticle targeting. The initial finding was the spontaneous build-up of macromolecular drug carriers in regions of solid tumors with leaky vasculature. The second discovery was the retention of nanoparticles within tumors as a result of poor lymphatic drainage. These discoveries led to the idea of EPR effect. Passive targeting and the EPR effect are closely related. To further comprehend this association, let us briefly review the pathophysiology of tumor vasculature, which allows for increased vascular permeability. Sustained angiogenesis, or the creation of new blood vessels and neovasculature, is a defining feature of metabolically active malignancy. This mechanism delivers nutrients, oxygen and helps to remove metabolic waste products from tumors. However, persistently stimulated angiogenesis can result in the creation of extremely aberrant tumor vasculature with chaotic, disordered courses and structural abnormalities from healthy arteries. Feeding arteries, mother vessels, glomeruloid microvascular proliferation, vascular malformations, capillaries, and draining veins are the 6 types of tumor blood vessels discovered by Nagy et al. [7]. These traits contribute to maternal arteries' exceptional permeability to plasma proteins and nanoparticles. Furthermore, tumor blood arteries, such as mother vessels and GMPs, may include inter-endothelial gaps, which increases tumour vascular leakiness. These inter-endothelial gaps enable intravenously injected nanoparticles to go from the lumen of tumor blood vessels to the tumour interstitium. This transport system, known as the paracellular route, is a passive process triggered by a concentration gradient between the blood and tumour interstitium.

The EPR effect implies that the administered nanoparticles can accumulate in solid tumors with discontinuous endothelium, provided they fulfil certain design parameters, such as being smaller than the cut-off size of tumor inter-endothelial gaps and having lengthy blood circulation periods. Longer nanoparticle blood circulation periods enhance the likelihood of paracellular nanoparticle extravasation (Fig. 2). Both active and passive nanoparticle targeting techniques use the EPR effect to deliver tumors by accumulating nanoparticles through concentration-dependent passive paracellular transport across discontinuous endothelial layers. Beyond solid tumors, the EPR effect has been detected in atherosclerosis, which is characterized by persistent inflammation of arterial blood arteries. This presents the concept of using nanoparticles to target regions of inflammation inside atherosclerotic tissue. Nanoparticles used to image and treat atherosclerotic plaques, which frequently feature microvasculature that permits nanoparticles put into the system to permeate. The endothelium of the blood vessels in the tumour tissues become more porous than that those of the healthy cells. Tumour cells during hypoxia grow new vessels and consume existing ones. The newly formed blood capillaries selectively let penetrate macromolecules larger than 40 kDa and nano-systems into the tumour stroma. Also, the nanoparticle retention is extended due to absence of lymphatic drainage. Exceptions to this are the small molecule medications which undergo instantaneous circulation and rapid clearance. Hence, the pharmacokinetics are improved by encapsulating small-molecule medications in the drug delivery systems. This entire method of targeting is known as passive targeting wherein the size and circulation time of the particle along with the tumour biology i.e. vascularity and leakiness is taken into consideration [8]. Passive targeting is mainly based on tumor biology (vascularity, leakiness) and carrier properties (size, circulation time). This method of tumor targeting lacks a particular ligand for certain types of tumor cells. The EPR effect relies heavily on fundamental tumor biology such as angiogenesis and lymph angiogenesis, perivascular tumor invasion, and intratumor pressure. These parameters, along with the physicochemical properties of nanoparticles, affect the efficacy of the particle drug delivery system.

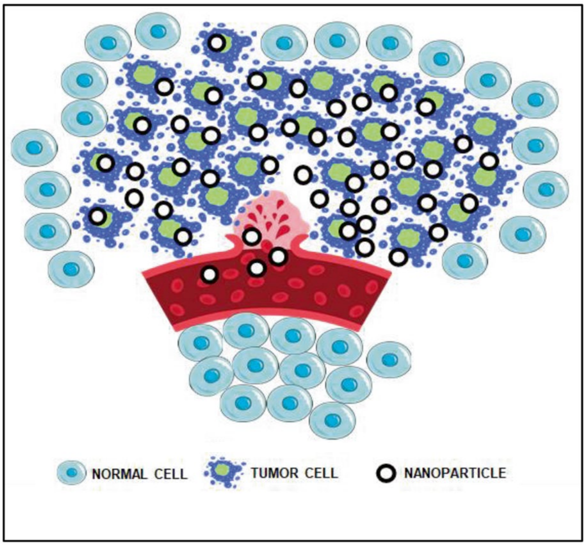

Fig. 2 Passive targeting of nanoparticles. Enhanced permeation and retention of nanoparticles at tumor site via blood vessels

Vascular normalization and enhanced nanocarrier accumulation can be seen when the EPR effect is modified either physically or chemically using enhancers like kinin, nitric oxide, peroxynitrite, prostaglandins, vascular permeability factor, vascular endothelial growth factor, and other cytokines producing hypertension or vascular normalization, which temporarily boosts tumor perfusion. Various techniques use ultrasound, radiation, hyperthermia, or photo-immunotherapy to target tumor vasculature and enhance nanosystem permeation. However, all stated approaches have limitations and contraindications that should be carefully considered.

Examples of Passive Targeting

Taxanes are one of the most effective medication classes used in cancer treatment. Paclitaxel (PTX) has demonstrated high potency against a wide range of malignancies. Taxanes are most commonly used to treat breast cancer, lung cancer (both small and non-small cell), and ovarian cancer. In 2005, the US FDA authorized Abraxane® (albumin-bound PTX, Abraxis Bio-Sciences), which is used to treat advanced or metastatic breast cancer [9]. Abraxane® is a medication that prevents microtubule depolymerization and hence stabilizes them. It occurs when the medication promotes microtubule assembly from tubulin dimers. Microtubule reconfiguration, which is essential for interphase and mitotic cellular activities, is inhibited by this enhanced stability. During the cell cycle during mitosis, the regularly used taxane PTX creates a distinct microtubule array and many asters. Abraxane® lowers pancreatic stroma in pancreatic cancer, either by alone or in combination with another cytotoxic medication like gemcitabine (GEM) xenograft mouse models [51]. Genexol PM® is a novel nanoformulation of PTX in a sterile lyophilized polymeric micellar formulation. According to experiments, Genexol PM® had a threefold higher maximum tolerated dosage in nude mice. Furthermore, the biodistribution was two- to three-fold higher in the liver, spleen, kidney, and lung, as well as in cancer cells. It is approved in South Korea to treat metastatic breast cancer. It is still under phase II clinical testing in the United States to treat pancreatic cancer [222]. DaunoXome® (liposomal daunorubicin; Gilead Science/Diatos) is an anticancer drug that inhibits tumor cell proliferation. The active ingredient is daunorubicin. It is a novel formulation of daunorubicin (in liposome form) used to treat Kaposi's sarcoma, a cancer of the skin, lungs, and gut. The US FDA authorized this in 1996 [223].

2.2 *Active Targeting*

For active targeting, nanoparticles contain specialized surface ligands. These surface ligands, also known as targeted ligands, might include biomolecules like proteins, nucleic acids, antibodies, and peptides. These chemicals can detect and attach to certain cell surface receptors present on malignant cells with great affinity. As a result, active targeting techniques in nanomedicine are purposely developed tactics that take advantage of unique biomolecular interactions between nanoparticle surface ligands and receptors on cell surfaces. The reason for active targeting is twofold. First, it seeks to increase the retention of passively collected nanoparticles at affected areas by leveraging the particular interaction between surface ligands and cell surface receptors. Second, it aims to improve nanoparticle specificity with affected cells while reducing interactions with non-targeted cells. This focused method improves the efficacy and specificity of nanoparticle-based treatments in cancer therapy.

Active targeting is based on specific ligands or chemicals, such as transferrin and folate, that bind to molecules or receptors specifically expressed or overexpressed on the target cells. This sort of targeting is known as ligand-mediated targeting. To achieve better affinity, nanoparticles containing ligands with specific activities such as retention and uptake, must be close to the target. This method improves the particle's binding to cancer cells, increasing medication penetration. Transferrin is one of the most investigated receptors. It is a type of serum glycoprotein that helps to transfer iron into cells. These receptors are overexpressed in most tumor cells and are expressed at lesser levels in normal cells. As a result, we can change the nanoparticles by adding related ligands that target transferrin. Targeting ligands, including proteins like transferrin, peptides like arginine-glycine-aspartic acid (RGD), vitamins like folic acid, and aptamers, can selectively deliver nanoparticles to specific cells or tissues.

Antibody-Targeted Nanocarriers

In order to create nanoparticle systems that actively aim at certain targets, antibodies, also known as immunoglobulins or antibody fragments like antigen-binding fragments and single-chain fragment variables are essential. Through weak interactions between antibodies and antigens, monoclonal antibodies can identify tumor antigens, enabling active tumor targeting [13]. Antibodies are 'Y' shaped proteins with two heavy and two light chains that bind to antigens/receptors on cancer cells, increasing payload absorption through carrier systems [229]. The design of antibodies-conjugated nanoparticles, including their configuration, origin, and manner of attachment, significantly impacts their efficacy in vivo. The most often employed antibodies in drug delivery nanosystems are anti-epidermal growth factor receptor for cancer cells and anti-vascular endothelial growth factor for tumors and lean vascular tissue [15]. Antibodies can be attached to nanoparticle surfaces in two ways: directly or via linker molecules like polyethylene glycol (PEG) [16]. Drugs like Gemtuzumab, Tositumomab, Ibritumomab, and Trastuzumab have been successfully used as nanocarriers in drug delivery systems, including liposomes, polymeric nanoparticles, cationic peptides, magnetic nanoparticles, carbon nanotubes, and mesoporous silica [17]. Magnetic nanocrystals and anti-HER2 monoclonal antibody trastuzumab selectively recognize HER2 in MCF-7 breast cancer cells.

The membrane tyrosine kinase receptor HER2 controls the processes of cell adhesion, migration, proliferation, and differentiation. In 25–40% of human breast carcinomas, it is overexpressed. Animals are used to produce and purify antibodies. Binding may be disrupted by animal differences, even within the same antibody. It has also been demonstrated that drug loading alters the characteristics of antibody recognition. In applications involving diagnosis and drug administration, aptamers, a stable, highly affinity ligand can take the role of antibodies [18].

Aptamers-Targeted Nanocarriers

By using SELEX (systematic evolution of ligands by exponential enrichment) to select short single-stranded DNA or RNA oligonucleotides or peptide sequences, aptamers can be produced as drugs or artificial antibodies. It is possible to repeatedly build aptamers to distinguish between cancerous and healthy cells [19]. Their non-toxicity and non-immunogenic properties make them ideal for concentrating on ligands with low molecular weights. They are more resilient and flexible than natural antibodies even after heat denaturation. These materials exhibit potential applications in the domains of biosensors, medication delivery, and cancer diagnosis and treatment. A variety of substances, including as proteins, amino acids, and pharmaceutical chemicals, are recognized by aptamers. Aptamers are built in the nanoparticle-aptamer conjugate system to bind high affinity and overexpress receptors on target cells. They fold into complex three-dimensional structures and are efficiently delivered to specific targets [14, 20]. Acute leukemia cells were specifically targeted with aptamer-conjugated magnetic nanoparticles and fluorophore-doped silica nanoparticles to identify intact cells present in mixed cell and whole blood samples. The use of very sensitive and selective biosensors to detect cancer cells has been the focus of recent research. Biosensors use aptamers as probes that interact with specific protein receptors on the cell surface. A biosensor-reported thiolated aptamer (thiol-TD05) is immobilized on Au-NPs. A biotinylated aptamer (biotin-TE02) is immobilized on the test zone of the aptamer-nanoparticle strip biosensor for the sensitive, rapid, specific, non-invasive, and inexpensive detection of the most common types of circulating cancer cells, including leukemia, lymphoma, and myeloma. It can bind to lymphocytic and myeloid leukemia. The biosensor detects the presence of gold nanoparticles in the test zone by a distinct red band, which can be evaluated visually or quantitatively [18].

Multi-walled carbon nanotubes (MWCNT) coupled with anti-prostate-specific membrane antigen (PSMA) aptamer were employed in another work to identify prostate cancer early on [224]. This nano-ultrasonic contrast agent was created by modifying the MWCNT using PEG and an anti-PSMA aptamer. An anti-PSMA aptamer with a 79 nucleotide 5′ alteration was combined with MWCNT-PEG to target prostate cancer cells since PSMA is abundantly expressed in metastatic prostate cancer cells offering high specificity and affinity. The dispersion, prostate cancer cell targetability, and visual quality of the nano-ultrasonic contrast agent were superior to those of the conventional contrast agents.

Folate-Targeted Nanocarriers

Drug conjugate systems that target folate receptors are often employed in developing anticancer medications. Folate, also referred to as folic acid, is vitamin B9 with a cell surface receptor anchored in glycosylphosphatidylinositol. Its small molecular weight (441 Da) reduces immunogenicity and renders it harmless, making it the perfect tumour-targeting ligand. It is also stable in circulation as well as can withstand various temperatures and pH levels [22]. Folic acid acts as a ligand targeting for small interfering RNA (siRNA) delivery methods and non-invasive disease diagnostics, including polymeric micelles, liposomes, macromolecules, carbon nanotubes, super paramagnetic iron oxide nanoparticles (SPION), and other nanoparticles [23]. Folate receptor is overexpressed in fast-growing human tumour cells, including epithelial malignancies of the ovary, breast, colon, lung, prostate, brain, kidney, myeloid, head and neck, and human nasopharyngeal carcinoma. The interaction between the folic acid ligand and folate receptor on the target cell surface leads to intracellular activation by receptor-mediated tumorigenesis. Specific endocytosis transports folate-conjugated drug nano delivery devices to endosomal vesicles [24].

In a study, a glycine linker attached camptothecin, an anticancer molecule, to PEG [25]. Folic acid was then linked to the opposite end of PEG as a spacer. Camptothecin is a cytotoxic alkaloid that disrupts the cell cycle during S-phase, promotes apoptosis, and inhibits DNA topoisomerase I activity (Fig. 3a). Long-term DNA topoisomerase I inhibition necessitates constant camptothecin levels in tumour cells. Camptothecin's short half-life and inadequate tumor targeting limit its long-term ability to lower DNA topoisomerase I. Because human nasopharyngeal cancer is characterized by overexpression of the folate receptor, a significant amount of camptothecin-glycine-PEG-folate bioconjugate is absorbed by the target cells. Through an endocytosis process particular to tumors, folate receptors facilitate the uptake of folic acid.

In another study, it was exhibited the efficacy of folic acid ligand targeting systems depends on the folate receptors' expression on target cells [26]. Folate-conjugated stearic acid grafted chitosan oligosaccharide micelles were tested for their capacity to target two cell lines (A549 and Hela) with varying folate receptor levels on their membranes. The A549 cell line (lung cancer) showed low uptake and negative expression of folate receptors on its cell membrane. In contrast, Hela cells (cervical cancer) showed high uptake and high expression of folate receptors. This study indicates that folate-mediated endocytosis correlates with the density of folate receptors on target cell membranes. Because folate receptors are not commonly found in pancreatic, prostate, testicles, bladder, and liver tumours, utilizing folate nanoconjugates for drug/gene delivery may not significantly increase uptake.

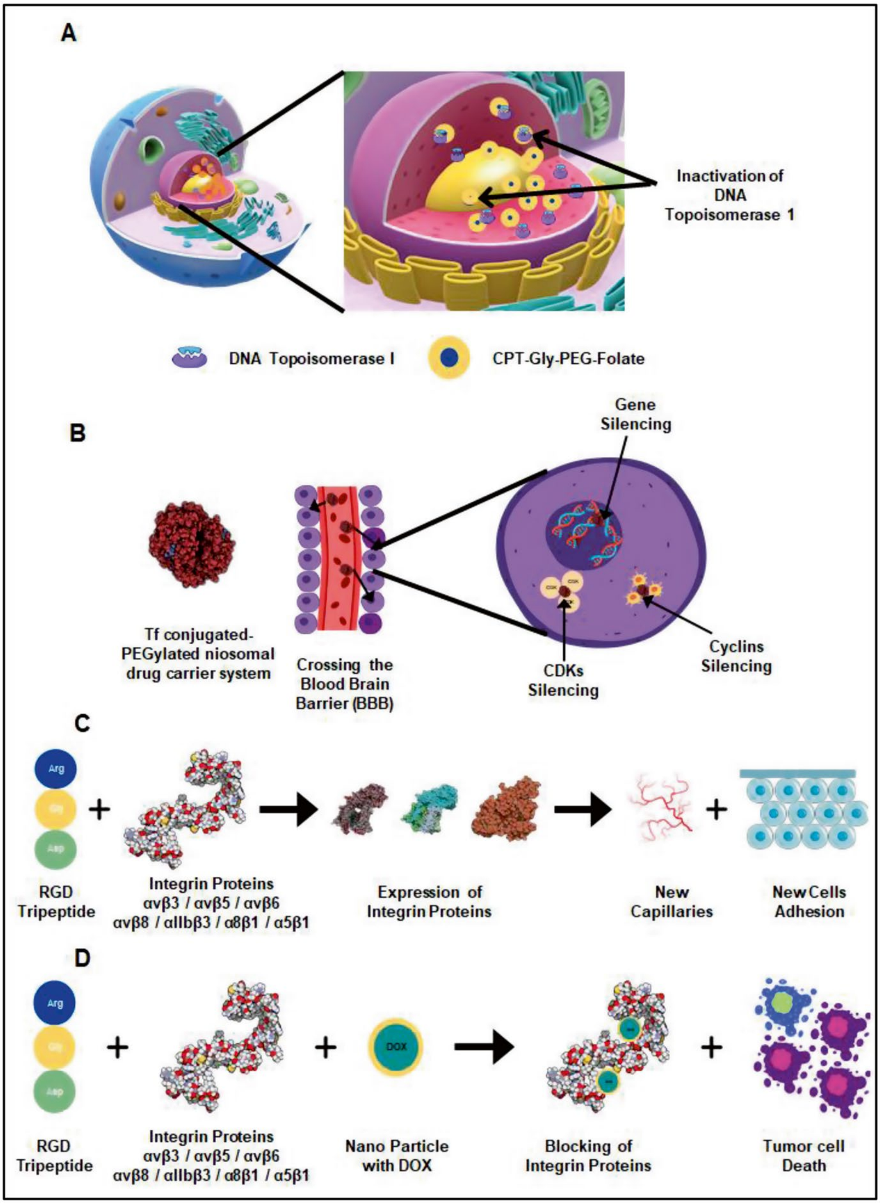
A
Inactivation of DNA Topoisomerase 1
DNA Topoisomerase I
CPT-Gly-PEG-Folate
B
Gene Silencing
Tf conjugated-PEGylated niosomal drug carrier system
Crossing the Blood Brain Barrier (BBB)
CDKs Silencing
Cyclins Silencing
C
Arg
Gly
Asp
RGD Tripeptide
Integrin Proteins αvβ3 / αvβ5 / αvβ6 αvβ8 / αIIbβ3 / α8β1 / α5β1
Expression of Integrin Proteins
New Capillaries
New Cells Adhesion
D
Arg
Gly
Asp
RGD Tripeptide
Integrin Proteins αvβ3 / αvβ5 / αvβ6 αvβ8 / αIIbβ3 / α8β1 / α5β1
DOX
Nano Particle with DOX
Blocking of Integrin Proteins
Tumor cell Death

Fig. 3 Active targeting of nanoparticles. (**a**) Targeting using folate (**b**) Targeting using transferrin (Tf) (**c**) Growth of new capillaries supported by RGD tripeptide (**d**) Targeting using RGD tripeptide (CPT camptothecin, Gly glycine)

Transferrin-Targeted Nanocarriers

The transferrin conjugated-PEGylated niosomal drug carrier system [27] enhanced cytotoxicity, accumulation in cell, and quicker and more thorough drug internalization. Using transferrin-targeted PEG-gold nanoparticles [28], the biodistribution and accumulation of nanoparticles in breast cancer cells were examined by contrasting their uptake with normal cells. The absorption of transferrin-gold nanoparticles is six times more than that of PEG-NPs, suggesting that the transferrin-receptor mediates cellular endocytosis. Normal cells absorb just around a quarter of that. This study discovered that transferrin nanoparticle absorption was dramatically reduced when transferrin receptors on target cells were blocked. Transferrin-targeted polymer nanoparticles filled with siRNA are used in gene silencing to stop the manufacture of a certain protein. Transferrin-targeted nanocarriers can inhibit cyclins and cyclin-dependent kinases (CDKs), which in turn inhibits hyperproliferative tumours and cancer cells by effectively arresting the cell cycle (Fig. 3b). Because of their low sensitivity and high contrast agent concentration, transferrin-conjugated nanocarriers are useful in diagnosis and monitoring systems such as magnetic resonance imaging (MRI) and computed tomography scan. A study found that using PEGylated, transferrin-conjugated liposomes loaded with improv (an iodine-based computed tomography scan contrast agent) as a theranostic system for glioma treatment and real-time delivery tracking computed tomography resulted in approximately fourfold increased contrast agent concentrations in glioma cells and prolonged contrast compared to untargeted liposomes [5, 18].

Tripeptide (RGD)-Targeted Nanocarriers

Arginine-glycine-aspartic acid (RGD) is a standard peptide that promotes cell adhesion in extracellular matrix. Proteins such as vitronectin, fibronectin, osteopontin, collagens, and fibrinogen, act as a ligand for integrins. Integrin proteins, including αvβ3/αvβ5/αvβ6/αvβ8/αIIbβ3/α8β1/α5β1 (also known as the fibronectin receptor), are heterodimeric transmembrane glycoprotein receptors with a high affinity for RGD sequences [29]. They facilitate angiogenesis and endocytosis by binding to receptors on cell surfaces or proteins in the extracellular matrix and regulate the cell cycle, development, invasion, growth, motility, and viability. Particularly, during angiogenesis, integrin αvβ3 is upregulated, which leads to an increase in the production of αv integrins on the membranes of endothelial cells. This encourages new capillaries to grow and survive (Fig. 3c). By directly influencing tumor cells, RGD-targeted nanoparticles that block αv integrin activity can slow down the development of tumors [30]. By using nanoparticles containing RGD peptide sequences, active targeting facilitates intracellular drug and agent delivery via receptor-mediated endocytosis. As an alternative, unique RGD-focused nano systems with various drugs or nanoparticles can block integrin function, inhibiting tumor-associated angiogenesis and metastasis. Overexpression of integrin receptors on tumor neovascular endothelial cells in ovarian, prostate, breast, and melanomas can lead to tumor growth and metastatic potential. RGD peptide-nanoparticles conjugates enhance medication and diagnostic imaging probe accumulation in angiogenesis-associated endothelial cells, reducing toxicity in healthy cells [31].

In a study, PEGylated gold nanoparticles were functionalized with cyclic 4-aminoproline-RGD semi peptides targeting αVβ3 integrins [32]. Nanoparticles compete for vitronectin binding to αVβ3 integrins, inhibiting human melanoma cell adhesion with a minimum inhibitory concentration (IC50) of around 1 nM. These nanoparticles are selectively internalized via receptor-mediated endocytosis. RGD is a suitable target for siRNA delivery for gene silencing, both in vitro and in vivo [33]. A study used RGD-PEG- poly(caprolactone) (PCL) as a siRNA (siLuc2) carrier to silence luciferase genes in vitro and in vivo. Compared to siLuc2 manufactured without cyclic RGD (PEG-PCL), the intravenous injection of RGD-PEG-PCL/siLuc2 effectively silenced lung metastatic cancer cells. Using dual targeting ligands such as folate and RGD peptide in a complex drug delivery system improves intracellular doxorubicin (DOX) accumulation and therapeutic efficiency, significantly inhibiting tumor growth in vivo. The chemical properties of RGD, such as its hydrophilicity, are important. In one study, the characteristics of cRGD peptides such RGDyC (Arg-Gly-Asp-D-Tyr-Cys) and RGDfK (Arg-Gly-Asp-D-Phe-Lys) targeting PEGylated liposomal DOX were investigated (Fig. 3d). Endocytosis mediated by receptors continues to be the principal mechanism for cell internalization. In a mouse tumor model of C-26 colon cancer, switching the target moiety from RGDfK to RGDyC decreased the liposomal system's hydrophilicity, which in turn led to reduced blood clearance rates and improved localization of PEGylated liposomal DOX [18].

3 Advancements in Different Nanoparticles

3.1 Polymer Based Nanoparticles

Synthetic and natural polymers are used in drug delivery. These include chitosan, gelatin, and albumin, which are natural polymers and PEG and polylactic acid, that are synthetic polymers. With high stability, mass production capability, it enhances anti-neoplastic activity with minimal side effects improving drug stability and specificity while reducing rapid metabolism and excretion. Usually, a blend of natural and synthetic polymers is used to exploit the properties of both for desired results. Wang et al. [34] constructed a pH-sensitive photothermal nanomaterial by self-assembling morpholine-modified silicone phthalocyanine with serum albumin which induced Type I reactive oxygen species (ROS) generation, enhancing immunogenic photodynamic therapeutic effect against hypoxic cancer cells. When combined with αPD-1-based immunotherapy, it inhibited cancer cell growth, reduced lung metastasis, and triggered tumor regression. Pham et al. [36] synthesized human serum albumin nanoparticles using albumin-binding technology with an PTX acid-sensitive linker conjugated to a monoclonal anti-PD-L1 antibody to the PTX-loaded serum nanoparticle, which resulted in enhanced drug release at low pH levels. When combined with cytotoxic T-lymphocyte antigen-4, the particles accentuated immune responses by increasing effector T-cell infiltration, immunosuppressive PD-L1, and regulatory T-cell suppression. Kim et al. [37] synthesized thermo-responsive hydrogel composed of gelatin and Pluronic® F127 for locoregional delivery of S-nitrosoglutathione (a nitic oxide-donor) and a monoclonal antibody targeting cytotoxic T-lymphocyte-associated protein-4. Improved local and systemic effects of cancer immunotherapy were seen along with the synergistic systemic co-delivery of the antibody and nitrosoglutathione. Bu et al. [38] developed a self-assembling gelatinase-sensitive nanoparticle comprising indocyanine green (ICG), a photosensitizer used in photothermal therapy (PTT), and NSC74859, a signal transducer activator of transcription 3 inhibitor where the combination of PTT with NSC74859-mediated immunotherapy leads to effective inhibition of head and neck squamous cell carcinoma tumor growth. The nanoparticles inhibit immunosuppression thereby improving anticancer efficacy. Wang et al. [39] synthesized hyaluronic acid-based macrophage-mediated delivery system by modifying thermal-conversion-based carbon nanoparticles with hyaluronic acid and loading with ICG, a photosensitizer which activated macrophages to release pro-inflammatory factors. This system upon irradiation, induces production of TNF-α and IL-2 in a 4 T1 tumor-bearing model, and generates ROS. Particle accumulation in the tumor was enhanced and it induced cytomembrane damage through ROS, apoptosis through heat, and pro-inflammatory cytokine-mediated immunotherapy. Yang et al. [40] synthesized a biomimetic vaccine using a pH-responsive chitosan-based polymeric micelle which promotes T-cell immune response induction and secretion of antitumor-related cytokines by directly delivering the antigen to the lymph nodes and facilitating uptake by dendritic cells. The pH-dependent property allows for precise control over antigen release and accumulation at the target sites. In a C57BL/6 mouse model, the micelle formulation effectively inhibits tumor growth by inducing higher levels of TNF-α, IL-12, and IL-6 indicating activation of immune cells and cytokine-mediated antitumor activity. Zhang et al. [41] synthesized a cyclodextrin-based supramolecularly assembled programmable immune activating nanodrug using poly[(N-2-hydroxyethyl)-asparagine]-Pt(IV)/β-cyclodextrin, cytosine-phosphate-guanine (CpG)/polyamido-thioxo-adamantane and methoxy poly(ethylene glycol)-thioxo-adamantane. In the tumour microenvironment, the high levels of ROS, promote nanodrug dissociation and the release of the payload which activates antigen-presenting cells and promotes antigen presentation and anticancer immune response. Chung et al. [42] synthesized fucoidan-based theranostic nanoplatform using fucoidan polymer, reducible polyamidoamine dendrimer, verteporfin, a photosensitizer and MnO_2 nanoparticles, serving as oxygen-evolving nanomaterials which showed synergetic photodynamic and triple negative breast cancer -targeted therapy, elicitation of an immune response, alleviation of hypoxia in tumours, inhibition of oncogenic signaling, reduction of cancer-mediated immunosuppression, enhancement of both anti-tumor immunity and anti-metastasis activity. Pei et al. [43] synthesized an antigen and adjuvant co-delivery platform for liver cancer immunotherapy using polyanionic alginate and polycationic polyethyleneimine to deliver glypican-3 peptide antigen and unmethylated CpG adjuvant. The electrostatic interactions between alginate and polyethyleneimine facilitate the delivery and promote antigen uptake and maturation by dendritic cells. In Hepa1–6 cells, the system induced cytotoxic T lymphocyte response leading to an increase in the infiltration of CD4+ helper T lymphocytes and cytolytic CD8+ T lymphocytes in the spleen. El-Deeb et al. [225] studied the immunotherapeutic effects of alginate/-carrageenan oral microcapsules containing MH751906, a compound from *Agaricus bisporus* on human colon cancer Caco-2 cells and found that microcapsules would activate natural killer cells that are resident in the gut followed by increase in the populations of CD16+CD56+ cells [225].

3.2 Biomimetic Nanoparticles

Biomimetic approaches are non-toxic and are based on "learning from nature" [44]. The main objective of the biomimetic nanoparticles is to incorporate the steric stabilization of polysaccharides, the immunological activity of proteins, the anchoring function of lipid bilayer and the drug-carrying ability of nanoparticle's core, into a single unified platform. These nanoparticles work similar to Trojan Horse approach for immune escape and prolonged circulation by a camouflage effect. One of the most commonly used biomimetic particles are liposomes that have shown excellent efficacy but have drawbacks of an incomplete membrane structure. Along with this, attempts to use asparaginase-loaded erythrocyte vectors for the treatment of acute lymphoblastic leukaemia are currently undergoing phase III clinical trial [45]. Problems faced in these systems are the passenger drug's biological abilities and sustained release of drugs.

Synthesis of liposome-based biomimetic nanoparticles includes a three step process *viz.* isolation of cell membrane-derived vesicles, synthesis of nanoparticles and fusion of membrane vesicles with the nanoparticles. The process of fabrication of cell membrane-derived vesicles from living cells is presented in the Fig. 4. Nanoparticles are prepared in parallel with the vesicles and are fused together to form the cell membrane coated nanoparticle (CMCNP) with a mean hydrodynamic diameter of 200 nm. Electrostatic interactions play an important aspect in fabrication of CMCNP. The cell membrane has negatively charged polysaccharides and sialic acid residues that maintain the integrity of the membrane and perform collective functions of the interactive space [46]. Hence, negatively charged NPs form a well-defined shell-core structure with membrane vesicles. In constrast, positively charged NPs tend to aggregate and possibly collapse the phospholipid bilayer and disrupt the local membrane structure. Unstabilized NPs, with higher surface energy, interact with cell membranes, leading to minimized energy and improved particulate stability. There are multiple strategies used for preparation of CMCNPs like co-extrusion method, sonication, microfluidic electroporation, electrostatic attraction, etc. Biomimetic cancer CMCNPs show stronger targeting capabilities because

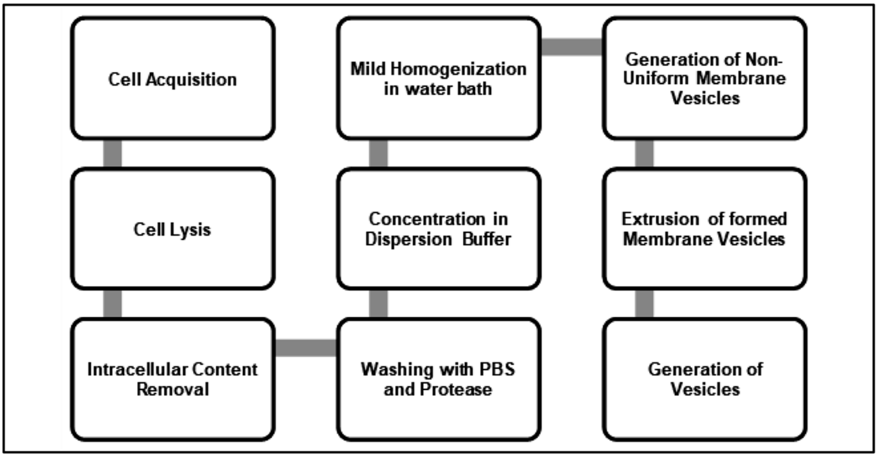
Cell Acquisition
Cell Lysis
Intracellular Content Removal
Washing with PBS and Protease
Concentration in Dispersion Buffer
Mild Homogenization in water bath
Generation of Non-Uniform Membrane Vesicles
Extrusion of formed Membrane Vesicles
Generation of Vesicles

Fig. 4 Method for fabrication of cell membrane-derived vesicles from living cells

of their multiple membrane receptors and protein corona formation, which enhances their effectiveness. Jan et al. [47] coated poly (lactic-co-glycolic acid) nanoparticles with MDA-MB-435 human breast cancer cell membrane, showing a stronger affinity for cultured MDA-MB-435 cells in vitro. In hepatocellular carcinoma cells, HepG2, the cell membrane-coated NPs showed active recognition and targeted delivery to tumors [48].

Red blood cells (RBCs) are the most abundant cellular component of the blood stream lacking nucleus and complex organelles. They also have intricate "self-marker" membrane proteins like CD47 which help to escape the immune surveillance. Shao et al. [49] utilized biomorphic poly (ethylene glycol)-block-poly(D,L-lactide) (PEG-PDLLA) for construction of polymersomes that closely resemble the RBCs. The dual compartment structure allowed co-encapsulation of hemoglobin and the photosensitizer coated with RBC membrane. This nanoparticle had enhanced EPR effect and was capable of producing oxygen radicals when exposed to light in the photodynamic therapy thereby addressing hypoxic tumor environments. Similar attempts for RBC membrane coated nanoparticles were made to encapsulate Fe_3O_4 and gold but did not meet the satisfactory efficacy for biocompatibility and selectivity. Apart from RBCs, leukocyte, monocyte/macrophages, lymphocyte, neutrophils, platelets and mesenchymal stem cell membranes have also been used to coat nanoparticles. Currently, attempts are being made to fuse multiple membranes together for gaining optimized properties. In an attempt, RBC membrane was fused with MCF-7 cell membrane and coated on melanin nanoparticle. The camouflaged particle showed increased circulation time owing to RBC membrane and homotypic targeting to MCF-7 cells [50].

Liposomal Nanoparticles

Liposomes are a lipid bilayer vesicle of phospholipids, which contain a hydrophilic core and can carry hydrophilic or lipophilic drugs that show high bioavailability, drug delivery, greater pharmacokinetics, and pharmacodynamics as well as antitumoral efficiency. As Matsumoto et al. [51] reported, the GEM-loaded liposome (FF-10832) manifested strong antitumor activity and prolonged shelf life in PC mouse xenograft models. Wang et al. [52] developed liposome with 'charge exchange' ability to actively transport GEM into tumor cells by ultrasound technology. Wang et al. [53] evaluated a redox reaction responsive liposome delivery system which allowed for higher loading of irinotecan with drug release activated by glutathione-induced redox reaction. Dwivedi et al. [54] showed that ultrasound pulses combined with DOX-loaded magneto-liposomes induced apoptosis in Panc-2 and BXPC-3 cell lines. Gao et al. [55] synthesized a microbubble-liposome complex carrying irinotecan and oxaliplatin in combination with ultrasound increased efficacy while tumours treated with this combination were 136% smaller and Phase I trials are showing promising results. Kimura et al. [56] used liposomes with surface-modified with rBC2LCN lectin (which binds specifically to fucosylated glycans found on pancreatic tumour cells) that showed decreased tumour weight in xenograft (Capan-1) mice. Duan et al. [57] synthesized liposome with cRGDfK peptide that responds to the enzyme MT1-matrix metalloprotease present on tumour cells and releases its drug load upon heat activation with controlled drug release and hindered tumour growth. Woo et al. [58] reviewed the use of nano-sized liposomal encapsulated irinotecan in combination with leucovorin-modulated fluorouracil (FU) which significantly improved the overall survival in patients and is gaining approval as as a second line agent for pancreatic cancer [59]. Xie et al. [60] developed nanoparticles of miRNA/siRNA which target both cancer cells and cellular interactions within the tumor stroma simultaneously, and also overcome compromised EPR effect in pancreatic cancer. Yu et al. [61] synthesized size-adjustable thermo- and fibrotic matrix-sensitive liposomes encapsulating BMS-202-loaded albumin nanoparticles to block immune checkpoints and showed attenuation of hypoxia and metastasis, enhanced T-cell activity, and increased secretion of interferon-γ and tumour necrosis factor-α in female C57BL/6 mice and Panc-2 cell lines. Xu et al. [62] synthesized PEGylated pH-sensitive liposomes carrying curcumin and GEM where, higher GEM concentration at the tumor site and increased cytotoxicity was observed. Wang et al. [63] used GEM-Mcl1 siRNA encapsulating cationic liposomes for co-delivery of GEM and siRNAs and reduced GEM resistance and enhanced anticancer effects.

3.3 Metal Nanoparticles

The major advantage of metal nanoparticles is that they can be used in cancer therapeutics, and diagnostics both *i.e.* theranostic approaches. These particles are synthesized either by top-down approaches using bulk materials or bottom-up approaches that utilized atoms and molecules. Metal nanoparticles are either noble (gold, silver, platinum) or non-noble (iron, copper, zinc, etc.). They allow for targeted and controlled drug delivery to tumors as well as detection and imaging of cancer site. In some cases, the metal particle is itself toxic to the cancer cells. These applications can be owed to easy fabrication and surface modification of the nanoparticles along with their optical and electromagnetic properties [64]. Few applications of metal nanoparticles are summarized in Table 3.

Table 3 Metal-based nanoparticles for diagnostics

Metal	Applications	Key findings	References
Noble metals	Photothermal therapy	Ultra-small-sized Pt nanoparticles showed strong photothermal properties and rapid tumor accumulation after functionalization with tumor-targeting agents Immense tumor regression observed after PTT with Pt nanoparticles Glutathione-functionalized ultra-small Pd nanosheets demonstrated strong optical absorption at 808 nm and high photothermal conversion rate. Complete tumor ablation was achieved in vivo using near infrared (NIR) irradiation at 808 nm.	[134, 135, 226]
Other transition metals	Photothermal therapy, Drug delivery, Photodynamic therapy, Multispectral optical tomography imaging	Ag_2S quantum dots generate sufficient heat in tumors and overcome therapeutic resistance of tumor cells by efficient PTT CuInS/ZnS quantum dots used for multispectral optical tomography imaging and combined photothermal/photodynamic therapy to overcome drug-resistant tumor cells Bi_2Se_3 quantum dots achieve enhanced cellular internalization and efficient antitumor efficacy in hepatic carcinoma cells due to their distinguished PTT Copper sulphide nanoparticles demonstrate strong NIR absorbance and high photothermal conversion rate for effective PTT	[136, 137]
Metal oxides	Photothermal therapy, MRI, Photoacoustic imaging	Fe_3O_4 nanoflowers serve as both photoacoustic and MRI agents for dual imaging and therapy Ti_8O_{15} nanoparticles exhibit potent IR absorption and high photothermal conversion efficiency for efficient PTT Black TiO_2 nanoparticles conjugated with anti-CD133mAbs and DOTA-Gd enable precise MRI-guided PTT for pancreatic CSC treatment	[138, 139, 227]

Platinum Nanoparticles

Pt nanoparticles are used for cancer diagnosis, therapy, and overcoming multidrug resistance in bacteria and tumor management by entering cells through passive diffusion or endocytosis exerting cytotoxic effects by introducing strand breaks in chromosomal DNA, leading to inhibition of replication, cell cycle arrest, and apoptosis. They inhibit cellular metabolic activity, generate hydroxyl radicals and release free Pt^{2+} ions and also act as antioxidants. These nanoparticles exhibit good photostability after multiphoton stimulation inside cells. López Ruiz et al. [65] fabricated a unique Ag-Pt nanoparticle which demonstrated high selectivity against melanoma and glioblastoma cells. No harmful effects were seen in normal fibroblast at doses ranging from 10 to 50 g/ml. The particles also exhibited substantial inhibition of pathogenic bacteria including *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus.* Gurunathan et al. [66] prepared Pt nanoparticles using tangeretin with combination of DOX, suppressed cell proliferation and viability, increased oxidative stress markers, induced mitochondrial dysfunction, and provoked DNA damage and repair mechanisms due to enhanced levels of 8-oxo-G and 8-oxo-dG in human bone osteosarcoma epithelial cells. Gurunathan et al. [67] tested the effects of Pt nanoparticles on exosomal and cellular function in human lung epithelial adenocarcinoma cells (A549 cells) which altered physiological processes leading to increased secretion of exosomes and promoted biosynthesis and secretion of exosomes through oxidative stress. Zhou et al. [68] fabricated Pt nanoparticles coated with phytic acid in combination with PTT for treating malignant bone cancers. These nanoparticles have a binding affinity for hydroxyapatite and bone fragments and high selectivity to accumulation in cancer related bone lesions inhibiting bone cancers growth and osteolysis. Fu et al. [69] prepared mesoporous Pt nanoparticles using F127 surfactant and coated them with PEG and loaded with DOX was taken up by drug resistant breast tumour cells MCF-7/ADR which showed enhanced cytotoxicity. When combined with photothermal and chemotherapeutic agents, it further demonstrated high efficacy against drug-resistant cancer cells compared to wild type. Mukherjee et al. [70] fabricated PEGylated Pt nanoparticles in colloidal form using borohydride reduction techniques to deliver DOX which suppressed tumour cell growth and stimulated apoptosis. When this system was administered intraperitoneally in a mouse model of subcutaneous melanoma, it showed significant tumour proliferation compared to free drug. The anti-tumour activity was confirmed by decreased expression of Ki-67 and SOX2 proliferation markers and increased expression of tumour supressing protein p53 in malignant melanoma.

Silver Nanoparticles

Ag nanoparticles can be synthesized in a straightforward and cost-effective way using green synthesis method. Silver also has biological characteristics like antibacterial and anti-fungal properties. Though they have low toxicity and poor absorption, they are internalized by cells through endocytosis, releasing Ag^{+} ions at target locations. They damage cancer cells by inducing the ROS, DNA Damage, apoptosis and necrosis [71]. They also modulate gene expression like p53, and signalling pathways like hypoxia-inducible factor pathway leading to cell cycle arrest and apoptosis and also inhibit tumour cell migration, angiogenesis and distant metastasis. Ag nanoparticles coated with unique protein covering called "Protein Crown" act as a nano-concentrator of serum proteins which is used in triple-negative breast cancer detection [72]. Ag nanoparticles are used as contrast agents because of their high optical absorbance and scattering ability and be detected in ultrasound imaging and photoacoustic. These nanoparticles target tumour sites especially, the leaky blood vessels. Ag nanoparticles conjugated with antibodies bind to antigens in tumour sites. Cheng et al. [73] used high sensitivity thermally annealed silver nanoparticle/porous silicon Bragg mirror composite substrate in combination with surface enhanced Raman spectroscopy approach to discover novel breast cancer biomarkers. Noorbazargan et al. [74] prepared Ag nanoparticles using *Juniperus chinensis* extract on human lung cancer (A549) tumor cells and found that these particles induce an increase in p53 expression which would lead to nuclear fragmentation, disintegration, and chromatin condensation, and eventually, increased ROS production. A decrease in cyclin D1 was observed in a great extent along with accumulation of cells in G0/G1 phase leading to apoptosis which was confirmed by upregulation of caspase-9 and caspase-3 and reduced the expression of MMP-2 and MMP-9. Faheem et al. [75] prepared Ag nanoparticles using *Bergenia ligulata* aqueous extracts; when tested on human breast adenocarcinoma (MCF-7) cells, it showed similar mechanisms. Gandhi et al. [76] reinforced with polyphenols extracted from lichen *Parmelia sulcata* were tested on MCF-7 cells decreased the levels of proliferating cell nuclear antigen and supressed the expression of TNF-α and IL-6 genes. Meenakshisundaram et al. [77] synthesized Ag nanoparticles using *Annona muricata* plant extract which reduced expression of cyclins B, E, and D which induced cell arrest and apoptosis in A549 cells. Singh et al. [78] synthesized using *Carica papaya* leaf extract were administered on human prostate tumour cells (DU145) and similar mechanisms. Hembram et al. [79] studied the effect of Ag and Au nanoparticles on tumour affected A549, A431, HeLa, HCT116, MCF-7, H-357, SSC-25, SCC-9 cell lines and non-tumour HEK-293, MCF-10A cells lines. The squamous cell carcinoma SCC-9 demonstrated changes in level of proteins for DNA repair, replication, and cell cycle regulation. The activity of Cdc-2 (Cdk1)/Cdk2 and cyclin A2 complexes were affected negatively whereas levels of checkpoint kinases (Chk1) and Cip/Kip proteins including p21 and p27 and the activity of Cdc25-A phosphatase were reduced. This all leads to cell cycle block in the S phase of the cell division cycle.

Ag nanoparticles induce apoptosis through intrinsic pathways which involves interactions between death receptors such as Fas, TNFR1, TNFR2, DR4, and DR5 and their corresponding ligands FAS, TRAIL, or TNF, initiator caspases (−8 and −10) propagate proapoptotic signals. Carneiro et al. [80] and Sur-Erdem et al. [81] synthesized Ag nanoparticles conjugated with TRAIL that sensitized TRAIL-resistant human glioblastoma cells and increased the activity of caspases (−3, −7, −8, and −9). Jiang et al. [82] tested a theranostic nanocomposite of Ag nanoparticles and peptide functionalized DOX on MCF-7/ADR cells activated the PERK (protein kinase R-like endoplasmic reticulum kinase)-mediated signalling pathway which phosphorylates eIF2α (eukaryotic initiation factor 2 alpha), ATF4 (activating transcription factor 4) and CHOP/GADD153 (growth arrest and DNA damage 153) regulates the apoptosis. Chen et al. [83] synthesizes Ag nanoparticles using polyvinylpyrrolidone-modification and administered in human prostate cancer (PC-3) cells showed significant upregulation of autophagy markers, decrease in number of lysosomes along with reduced protease activity and downregulation of cathepsin D. Over the last decade, researchers have demonstrated the promise of nanosized materials in cancer therapy, with Ag nanoparticles being the most often employed due to their antibacterial characteristics and distinct cytotoxic effects [84, 85]. However, questions regarding long-term toxicity persist. To completely comprehend its impacts on cells, cancer tissues, and organs, extensive nanoparticle characterisation and conventional experimental design are required.

Gold Nanoparticles

Since the past few decades, Au nanoparticles have gained immense attractions in nanomedicine due to their therapeutic effects and low toxicity. The surface of the Au nanoparticles is extended by modification with cationic polymer chains or functional groups like carboxyl, amine, or thiol groups which also stabilize the medications. Immune-therapeutic polymers like fucoidan have also been used to produce biocompatible Au nanoparticles with reduced toxicity. Apart from being extensively used as a delivery vehicle of chemotherapeutic agents, Au nanoparticles have considerably been used for delivery of nucleic acids acids as well. Some recent studies of nucleic acid delivery using Au nanoparticles are reported in Table 4. Au nanoparticles have been synthesized in different formats like rods, shells, spherical particles, etc. Like other metal nanoparticles, Au nanoparticles have been used for both cancer therapeutics, diagnostics as well as theranostic purposes [86]. Other than targeting cancer cells, Au nanoparticles in different formats have also been used to aim cancer stem cells (CSCs); the corresponding studies are summarized in Table 5.

Magnetic Nanoparticles

Magnetic nanoparticles are type of metal-inorganic nanoparticles that can be manipulated using magnetic field due to their ferro and super magnetism. They produce heat generating magnetic fields and hence used in hyperthermia cancer treatment, controlled drug release, MRI, and biosensing. They are used as contrast agents for MRI for cancer detection and monitoring. Upon irradiating magnetite (Fe3O4) nanoparticles, they efficiently convert light into heat and induce apoptosis in cancer cells and combining with immunotherapy can stimulate further tumor shrinkage and reduce the risk of recurrence and metastasis [87]. Super magnetic ferrite particles, SPIONs and manganese ferrite nanoparticles, are functionalized with targeting

Table 4 Gold nanoparticles as delivery systems for nucleic acids

Cargo	Delivery system	Method	Outcome/Result/Effect	References
DNA	Bcl-2 DNAi-conjugated Au nanoparticles	Functionalization with gene-silencing DNA	Potential for clinical applications	[140]
	Crotamine-functionalized Au nanoparticles	Functionalization with cell-penetrating peptide	DNA delivery potential	[141]
	Y-type DNA-functionalized Au nanoprobe	Surface modification with beacon structure	Telomerase identification and drug release triggering	[142]
RNA	Au-Fe oxide nanoparticles surface loaded with therapeutic micro RNA	Nose-to-brain direct transport pathway	Promising results in glioblastoma treatment	[143]
	Multilayered nanoparticles carrying miR780	Localization to target cancer area	Potential for reducing lung metastasis in triple negative breast cancer	[144]
siRNA	Glucose-installed Au nanoparticles	Targeted delivery to breast cancer cells	Efficient silencing of target gene in breast CSCs cells	[145]
	HIV-1 TAT peptide-capped Au nanoparticles	Conjugation with targeting peptide	Potential treatment for invasive breast cancer	[146]

ligands such as folic acid or antibodies. Ultra small SPION provide brighter vascular images. Magnetic hyperthermia is a nanoparticle mediated thermotherapy in which the particles act as nano-heaters that converts applied magnetic fields into thermal energy. Magnetic nanoparticles injected as colloidal suspensions accumulate passively by the EPR effect or actively by surface ligands. Nanoparticles used in magnetic hyperthermia must exhibit super para magnetism with zero magnetization in absence of external magnetic field to prevent aggregation. The shape of nanoparticles affects the effectiveness in hyperthermia like rod has greater magnetic torque and hence more effective than spherical nanoparticles. Kulikov et al. [88] synthesized oleic-stabilized iron oxide nanoparticles for anti-tumor hyperthermia therapy, achieving efficient hyperthermia with no toxicity and high survival rates in animal models. Gandhi et al. [89] fabricated cobalt ferrite nanoparticles for bimodal hyperthermia and their mechanistic interactions with lysozyme for magnetic hyperthermia therapy increasing local heating temperature. Suleman and Riaz [90] used $CoFe_2O_4$@$MnFe_2O_4$ i.e. core shell magnetic nanoparticles for magnetic hyperthermia for liver cancer cells using computer-based simulations. Taherian et al. [91] used chitosan functionalized SPION as a drug carrier for black pomegranate peel extract as a novel anti-cancer drug for breast cancer treatment with good loading efficiency and no toxicity. Jędrzak et al. [92] used polydopamine coated iron oxide nanoparticles enveloped with polyamidoamine for theranostic action on liver cells and when combined with chemotherapy and PTT, they activated cell apoptosis. Further research must be focused to investigate the longterm toxicity of magnetic nanoparticles by optimizing their shapes, sizes and structures and developing mechanisms for effective delivery into inner body parts during external magnetic field.

Table 5 Nanomaterial systems in liver cancer treatment

Reference	Nanoparticle system	Purpose/features
[147]	Targeted functional MRI contrast agent (12,12-NB-DTPA-Gd) liposomes	Exploitation of the EPR effect for tumor accumulation, triggered drug release via internal glutathione and external UV stimulation.
[148]	Double-reduction/pH-responsive nanocarrier	Responsive to both reduction and pH changes in the tumor microenvironment, delivering capsaicin and DOX.
[149]	Curcumin and dasatinib loaded pH sensitive micelle	Selective transport and targeting of tumor cells for cancer treatment, efficient loading and delivery of multiple drugs.
[150]	pH/NIR dual-responsive hollow mesoporous silica nanoparticles	Co-delivery of DOX and ICG with pH and NIR responsiveness.
[151]	Dual-pH and thermosensitive copolymer hydrogel	Formulated for controlled drug release in response to changes in pH and temperature, based on *N*-isoproplylacrylamide and acrylamide monomers
[152]	Dual-targeted nanoscale drug delivery system	Utilizes EpCAM aptamers and lactic acid-modified oligoamide amine dendrimers for simultaneous delivery of disulfiram and ICG, enabling imaging and therapeutic functions.
[153]	Dual-gated folate functionalized nanodiamond delivery system	Features activated fluorescence and cytotoxicity for DOX, controlled via dual gating mechanisms and folate targeting.
[154]	Dual-stimulus poly(*N*-isoproplylacrylamide-*co*-acrylamide crosslinked with melamine-glutaraldehyde and loaded with curcumin	Designed for temperature-responsive and pH-induced drug delivery, potentially applicable in controlled release chemotherapy.
[155]	Double-response and photoluminescence nano-gel comprising carbon quantum dots/β-cyclodextrin/poly *N*-isoproplylacrylamide@ poly(acrylic acid)- D-galactose	pH and temperature-responsive nano-gel with photoluminescence, evaluated for targeted drug delivery.
[156]	Multifunctional coordination nano-prodrug (hyaluronan/cisplatin/ICG)	Utilizes cisplatin hydrazide and cisplatin ICG coordination reaction, enabling multifunctional drug delivery.
[157]	Phosphate peptide-modified polydopamine-encapsulated nanoparticles (hollow mesoporous silicone DOX)	Constructs nanoparticles for the targeted delivery of DOX to cancer cells, utilizing phosphate peptide modification and polydopamine encapsulation.

(continued)

Table 5 (continued)

Reference	Nanoparticle system	Purpose/features
[158]	Survivin siRNA@T7- Aggregation-Induced Emission Gd nanoparticles	Novel siRNA vector nanoplatform for targeted and real-time monitoring of hepatocellular carcinoma, with dual-mode imaging characteristics.
[159]	Cholic acid-, galactose-, or lactose-Bi-conjugated chitosan derivatives	Potential anti-liver cancer drug carriers characterized via various spectroscopic and morphological analyses.
[160]	Porous degradable polymer microspheres	Hypoxic-activated prodrug delivery system for the combined release of DOX and dexamethasone into the liver.
[161]	Multifunctional Trojan horse-like amphiphilic EPR-independent nanobowls	Unique nanostructure for separate storage and release of hydrophobic sorafenib and hydrophilic DOX drugs under pH/NIR dual-stimuli.
[162]	Multifunctional liposomes	Novel liposomes for co-administration of curcumin and capsaicin with target ligands (glycyrrhetinic acid and galactose) for enhanced efficacy.
[163]	Liver cancer-specific mitochondria-targeted double-linked metal organic scaffold	Designed for sequential drug delivery to cells and mitochondria in liver cancer.
[164]	Fe_3O_4 nanoparticles coated with polymer carrier and loaded with 5-FU	Utilization of nanoparticles for magnetic targeting and delivery of FU-based drugs to cancer cells.
[165]	Aminated mesoporous silica nanoparticles and corn prolamin-based dual-drug carrier	pH-responsive delivery system for co-delivery of 5-FU and curcumin to liver cancer cells.
[166]	Double-ligand liposomes modified with enoxolone and cRGD	Dual-targeting liposomes for improved anti-tumor efficiency via glycyrrhetinic acid receptor and α-β integrin targeting.
[167]	DOX-conjugated single-wall carbon nanotubes functionalized with mannose	Functionalized nanotubes for targeted delivery of DOX to liver cancer cells.
[168]	Ethyl cellulose-based microspheres for dual-loading of $NaHCO_3$ and DOX	Microspheres for synergistic trimodal interventional therapy by pH modulation in the tumor microenvironment and enhanced inhibition of tumor cell growth.
[169]	Multi-component microsphere system with co-encapsulation and spatiotemporal drug release capabilities	Postoperative treatment of cancer and liver regeneration using microspheres loaded with DOX and liver regeneration-enhancing factor with controlled release.
[170]	Dual-pH-sensitive intelligent nanocarrier based on silica nanoparticles coated with chitosan and loaded with DOX	Inhibition of liver cancer cell proliferation using dual-pH-sensitive nanocarriers with homologous targeting ability.

Zinc Oxide Nanoparticles

ZnO nanoparticles are effective in cancer treatment, medication delivery, and other therapeutic uses due to their small size, efficient absorption by the human body, and their high biocompatibility. They have also been investigated as carriers for drug, gene, protein, and imaging agent, particularly for tumor-specific medication administration. Ramesh et al. [93] used Au-doped ZnO nanorods coated on the interdigitated electrode chip surface with viral DNA receptors that resulted in high sensitivity for detecting HPV-16-E6 oncogene targets at extremely low concentrations (1fM). Sundraraman and Jayakumari [94] synthesized a biodegradable polymer-coated ZnO nanoparticles loaded with taxifolin which demonstrated high efficacy against cancer cell lines, particularly inhibiting MCF-7 cells even at a lower dose. These nanoparticles induced apoptotic signals leading to nuclear fragmentation. George et al. [95] developed nanohybrid hydrogels using L-histidine conjugated chitosan with embedded ZnO nanoparticles for delivering polyphenol drugs such as naringenin, quercetin and curcumin. Histidine-chitosan conjugation stabilizes the hybrid hydrogel and facilitated long-term drug administration at pH 5. Zhou [96] synthesized ZnO-SiO_2 core shell nanoparticles, coated with poly ethylamine and surface-functionalized with cholic acid which efficiently deliver Ru pro-drugs in cervical cancer treatment and exhibited a greater ability to produce ROS. Sathishkumar et al. [97] synthesized a ZnO nanocomposite loaded with quercetin that showed faster release of quercetin targeted at MCF-7 cancer cells.

Copper Nanoparticles

Cu ions modulate overall survival in cancer tissues by initiating the Fenton reaction, which produces ROS [98]. Cu, unlike other transition metals, has a faster reaction rate and can function across a wider pH range, making it a powerful inducer of ROS by the formation of hydroxyl radicals (·OH). Cu-induced apoptosis is an important technique for targeting cancer cells. Excessive Cu levels can increase ROS levels, causing mitochondrial damage and activating the mitochondrial apoptosis pathway [99]. This process involves the regulation of pro- and anti-apoptotic proteins, which leads to mitochondrial malfunction, cytochrome C release, and caspase cascade activation, respectively. Furthermore, Cu-induced ROS can cause endoplasmic reticulum stress-mediated apoptosis, which contributes to cell death [100]. Cu-induced ROS has been linked to protective autophagy, which can either protect cancer cells from death or make them susceptible to apoptosis or other forms of cell death. Furthermore, Cu can cause ferroptosis, which is a type of iron-dependent cell death characterized by lipid peroxidation [101]. Copper-based medicines have been demonstrated to trigger ferroptosis in cancer cells by affecting iron homeostasis and boosting lipid peroxidation, making them a prospective option for cancer treatment [102]. Copper, in addition to apoptosis, autophagy, and ferroptosis, can cause pyroptosis, a caspase-1-dependent kind of programmed cell death linked to inflammation. Copper-induced ROS can activate the NLRP3 inflammasome, resulting in pro-inflammatory cytokine production and, ultimately, pyroptotic cell death [103]. Furthermore, copper-based complexes have been demonstrated to cause paraptosis, a caspase-independent kind of cell death characterized by mitochondrial and/or ER dilatation. Copper-based therapy can induce ER stress and modulate the ubiquitin-proteasome system, triggering paraptosis in cancer cells and providing a viable way to overcome apoptosis resistance [104]. Furthermore, a new type of copper-dependent cell death called cuproptosis has just been discovered. Intracellular copper build-up causes lipoylated mitochondrial enzyme aggregation and loss of Fe-S proteins, ultimately leading to cell death [105].

4 Advancements in Nanomaterial-Based Treatment in Different Cancers

Nanomaterials have been vastly and extensively employed for treatment and diagnosis of cancer. Almost all cancer types have been targeted using different types and format of nanomaterials. In this section, we have focussed on recently reported studies in major cancer types.

4.1 Liver Cancer

Hepatocellular carcinoma is now the fifth most common cancer worldwide and is the third most common cause of cancer mortality globally. HCC normally occurs in the background of chronic liver injury and fibrosis; mutational events in genes such as TP53 and CTNNB1 contribute to its malignant transformation. Symptomatic and asymptomatic stage A liver cancer can be eradicated through surgical resection, liver transplantation, or local treatments such as radio frequency ablation. However, the disease is often diagnosed at an advanced stage, and most cannot be cured. In the late stages of liver disease, systemic treatments include sorafenib and lenvatinib for cancer therapy or immunotherapy with tested immune-control points. The different strategies employed for liver cancer using nanomaterials are summarized in Table 5. The three top and effective measures for preventing liver cancer are through large-scale vaccination for Hepatitis B, treatment Adoption of antiviral for Hepatitis C and general screening for the atrisk population. However, as mentioned before, the early diagnosis is still a major problem because this disease is mostly silent in its majority of the progression and may only become symptomatic in its later stages; therefore, the question of biomarker identification and better diagnostic methods remains open.

4.2 Cervical Cancer

Cervical cancer still continues to be a major cause in women despite there being widespread preventive measures of early examinations and vaccinations. The disease is mainly associated with chronic exposure to high risk of HPV types, including HPV-16 and HPV-18, through which cervical epithelial cells transform to malignant type. Untreated cervical cancer develops from asymptomatic abnormal cells called cervical intraepithelial neoplasia (CIN) to invasive carcinoma. The two main types based on histological structure are however the squamous cell carcinoma and the adenocarcinoma. While locally confined cervical cancer may be managed by surgery and radiotherapy in the initial stages, advanced cervical cancer can only be treated by chemotherapy and radiation, with less satisfactory therapeutic results. HPV vaccines have played a critical role in reinventing the cervical cancer that has reached out across the world, regular Pap smear tests and screening has lowered the incidents and mortality rates. However, there are barriers in implementing the preventive measures in that people in the rural area have less access to these essentials, more research needs to be done for cervical cancer preventive measures. Table 6 includes information of different nanoparticle systems used for cervical cancer treatment.

Table 6 Nanoparticles systems in cervical cancer treatment

Reference	Study	Characteristics and results
[171]	Andrographolide-loaded nanofiber membrane	Sustained in desired concentration following a first-order pattern for 3 weeks. Decrease in tumour size Absence of inflammation or tissue necrosis. Negative correlation between drug content and mechanical strength. Better release observed with 6:1 polymer-to-drug ratio compared to 3:1 ratio.
[172]	Co-loaded DOX and ICG into mesoporous silica nanoparticles incorporated into nanofibers	Kills more cells upon laser irradiation for 10 min. High-intensity fluorescence Tumor inhibition rate was 38% in vivo.
[173]	Core–shell nanofibers integrated with MWCNT and 5FU	Significant higher cytotoxic in HeLa cells.
[174]	DOX-loaded core–shell pH-responsive nanofibers	Drug released initially in a burst then gradual release pattern. Apoptosis observed in HeLa cells after 3 days of treatment.
[175]	GEM-loaded transvaginal chitosan-lecithin hybrid nanoparticles	The hybrid particles show initial burst release of 70% in first 2 h, sustained release for 24 h. GEM-hybrid nanoparticles cause significant increase in cervical superoxide dismutase content and decrease in malondialdehyde and interleukin-1β content.

(continued)

Table 6 (continued)

Reference	Study	Characteristics and results
[176]	Aptamer AS1411 conjugated with Au nanoparticles	Enhanced uptake of model drug Initiated faster antiproliferative activity (in 3 days), in HeLa and HEC-1-A cell lines.
[177]	Neo-poly gramicidin mixture and Bi lipophilic nanoparticles	Combination of 0.3 mg/mL gramicidin with 10 µM nanoparticles results in 86% cell growth inhibition and antitumor activity on HeLa cells.
[178]	Ag nanoparticles from snail mucus	Cytotoxic effect in seen most effective at 64.05 µg/mL in HeLa cells.
[179]	In situ gel containing toad venom entrapped solid lipid nanoparticles and micronized nanorealgar	Inhibition of HeLa and SKOV-3 cell growth Optimum gel composed of 27.5% Pluronic F127 and 5% Pluronic F68, with gelation temperature of 33 ± 0.91 °C. Long-duration bioadhesion observed in vivo. No local inflammation with intact vaginal mucosa and epithelial lining.
[180]	In situ hydrogel containing nanocrystals coated with dopamone for imatinib delivery	Particles showed positive charge and high drug content. Faster release and enhanced inhibition of cell growth and apoptosis.
[181]	Phytantriol-basedi in situ gel for vaginal delivery of sinomenine hydrochloride	Initial burst release followed by sustained drug release up to 144 h.
[182]	Aloe-loaded alginate-based hydrogel	Comparable inhibition of cancer growth to standard chemotherapeutic agents.
[183]	Self-assembled hydrogel from raltitrexed for prevention of postoperative tumor recurrence	In vitro release profile showing initial fast release followed by slowing down for next 27 days. Cytotoxicity observed in HT29 and HeLa cells while maintaining cell viability in L929 cells.
[184]	Carboplatin-loaded nanosponge-containing hydrogel	Sustained release of carboplatin observed over 24 h and enhanced cytotoxic activity.
[185]	Nanogel self-assembled from raltitrexed for vaginal delivery	Successful cellular uptake of raltitrexed observed, comparable cytotoxicity, high binding ability to mucin. Nanogel showed highest tumor-inhibiting ability.

4.3 Pancreatic Cancer

Pancreatic cancer is an extremely lethal form of cancer that until recently was largely diagnosed at a very late stage because its early manifestation is often asymptomatic. It contributes to one of the highest cancer related mortality indexes around the globe due to factors such as chemoresistance, and high rate of metastatic progression. The first type of pancreatic cancer is the pancreatic ductal adenocarcinoma, the cancer originating in the exocrine gland and is linked to genetic mutations including KRAS, TP53, and CDKN2A. Today there are mainly surgical, chemotherapy and radiation therapy but they are not so effective as it is necessary to find new targets of treatment. There is a marked shift in research towards identifying molecular-based treatment, using the immune system to combat cancer, and ways of screening in order to enhance the Food and Drug Administration rates. The problem of early diagnosis, however, persists due to the absence of reliable biomarkers, as well as the localized position of the pancreas so the development of new diagnostic approach becomes essential for improvements in the treatment of the patients. Table 7 discusses various types of cancer treatment for pancreas over the last few decades.

Table 7 Nanoparticles systems in pancreatic cancer treatment

Authors	Study	Key findings
[51]	Liposome (FF-10832) encapsulating GEM	Enhanced long-term stability of GEM in plasma and better antitumor activity.
[186]	Collagenase-loaded liposomes followed by PTX-loaded micelles	Improved drug penetration into the pancreas by disassembling dense extracellular collagen stroma matrix Enhanced treatment effectiveness
[187]	Bispecific antibody-targeted liposomal irinotecan	Efficient drug encapsulation, loading and cellular uptake and inhibition of cancer growth in vivo
[188]	Investigation of cytotoxic effect of GEM encapsulated in different solid lipid nanostructures	Higher cytotoxic effect observed on primary pancreatic cancer cell lines and MiaPaCa-2 pancreatic cell lines Nanoparticles improved therapeutic effect of GEM on pancreatic cancer cell lines
[189]	PTX encapsulated in albumin nanoparticle integrated into modified thermosensitive liposome	PTX retention in solid tumor enhanced by incorporating it into liposome. Addition of photothermal agent IR-780 widened tumor's interstitial space Combination of PTT with nanoparticle administration promoted release of drug system encapsulated within modified liposomes.
[190]	SPION encapsulating curcumin with GEM	Effective delivery of curcumin by SPION into tumor cells Enhanced uptake of GEM and its efficacy Decreased tumor sphere formation with co-treatment of encapsulated curcumin and standard GEM Elevated levels of curcumin in pancreas and reduced tumor growth and metastasis observed in model pancreatic cancer mice
[191]	Folic acid-conjugated SPION loaded with ovinblastine	Low cytotoxicity against healthy cells and high apoptosis in PANC-1 pancreatic cancer cell lines observed Prevention of cell proliferation in PANC-1 cells
[192]	Mesoporous silica nanoparticle for co-delivery of GEM and cisplatin	Overcoming resistance to platin-based drugs and suppressing tumor growth Removal of off-target toxicities of chemotherapy combination
[193]	PEGylated Au nanoparticles for combination therapy of DOX and varlitinib.	Encapsulation of DOX and varlitinib into PEG-Au nanoparticles reduced toxicity against healthy pancreatic cell lines while increasing toxicity against S2-013 pancreatic cancer cell line.
[194]	Liquid metal mixture-based nanoparticles.	Liquid metal mixture-based nanoparticles conjugated with benzoporphyrin derivative and hyaluronic acid demonstrated improved cellular uptake, targeting competence, and significant intracellular ROS generation, leading to effective destruction of cancer cells upon NIR light activation. Tumor regression and higher necrosis.

4.4 Lung Cancer

Lung Cancer has the highest mortality rate in both men and women. Around 85% of them are non-small-cell lung cancers (NSCLC), and rest are small-cell lung cancers. Advancements in screening, radiation and treatment have led to a decline in NSCLC mortality and overall decrease in lung cancer deaths. Increased screening has contributed to earlier diagnosis and treatment but there is low 5-year survival rate among older patients with stage IV NSCLC. Various drugs used to treat conditions such as hypertension, hyperlipidemia, inflammation, diabetes, and infections are also used in nanoparticles as a part of nanotechnology treatment for NSCLC. MRI guided nanoparticle therapy are used for detection of cancer with high EPR effect. Liposomes are used for delivering siRNA containing cationic lipids with the tissue factor pathways inhibitors suppress lung metastasis. Soni et al. [106] developed mannosylated solid lipid nanoparticles using emulsification and solvent evaporation techniques surface-modified with mannose using the ring-opening method and loaded with GEM. GEM-loaded particles exhibited higher cytotoxic effects on A549 lung cancer cells due to the uptake of targeted nanoparticles by cancer cells via mannose receptor-mediated endocytosis. Mottaghitalab et al. [107] synthesized silk fibroin nanoparticles targeted with the SP5-52 peptide loaded with GEM showed a higher toxic effect on the proliferation of LL/2 cells compared to non-targeted nanoparticles and free drug. Computed tomography analysis of tumors in LL/2 lung tumor-bearing mice treated with GEM-loaded targeted particles showed a greater reduction in tumor size. Further, whole-body imaging confirmed higher accumulation of the targeted nanoparticles in the lungs compared to other tissues. Feldmann et al. [108] studied virus-inspired polymer for endosomal release composed of a polycation segment for nucleic acid condensation and a pH-sensitive segment for uncovering the lytic peptide existing in the membrane to induce endosomal escape. It used to carry siRNA into lung cancer cells showed acceptable internalization into NCI-H1299 cancer cells in vitro. When introduced intrathecal into mice, it exhibited high accumulation in bronchioli and alveolar epithelia compared to unmodified block copolymer polyplexes suggesting that virus-inspired polyplexes are capable of escaping from the acidic microenvironment of the endosome and safely delivering siRNA into the cytoplasm of lung cells with a neutral pH, making it an effective and safe route for delivering cargos into cancerous lung tissue. Mandal et al. [109] synthesized hybrid nanoparticle system termed CSLPHN containing a biodegradable PCL core and a shell composed of hydrogenated soy phosphatidylcholine and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N [methoxy(PEG)-2000 monolayer to deliver erlotinib, an anticancer agent, into NSCLC cells. Post optimization, erlotinib showed encapsulation efficiencies ranging from 18% to 66% w/w and loadings of 1.6% to 2.1% w/w on CSLPHNPs and approximately 50% of the drug was released during the first 3 h, which decreased after 24 h at pH 7.4. Song et al. [110] evaluated the properties of epidermal growth factor receptor-targeted lipid-polymer nanoparticles containing stearic acid and polylactic acid for the co-delivery of docetaxel and resveratrol and found that conjugating the receptor to the nanoparticles enhanced the endocytosis of nanoparticles into the cells. Li et al. [111] used nanoparticle called transferrin conjugated PEG-hydrazone-glyceryl monostearate solid lipid nanoparticles loaded with docetaxel and baicalein. The transferrin-decorated nanoparticles were specifically targeted and internalized into the cancer cells and the PEG layer degradation improved the release of both the drugs. Guo et al. [112] synthesized poly lactic-co-glycolic acid nanoparticles coated with lipid and conjugated to transferrin. The particles were loaded with DOX and controlled the release of DOX over time intervals was achieved, only approximately 65% of DOX was released within 3 days. The lipid-coated nanoparticles conjugated with transferrin also exhibited higher cytotoxicity and more efficient endocytosis into A549 cells.

4.5 Skin Cancer

Cancer of the skin is one of the most widespread forms of cancer around the world and incidences are increasing as a result of exposure to ultraviolet light from the sun or artificial sources such as sunbeds. The most common types of skin cancer include basal cell carcinoma, squamous cell carcinoma and melanoma; melanoma being the most dangerous skin cancer kind. Basal and squamous cell carcinoma that are referred to as non-melanoma skin cancers are usually less aggressive and have a high likelihood of being treated in case they are diagnosed early. Melanoma commonly originates from melanocytes is highly invasive and usually associated with BRAF/NRAS gene mutation. That is why the identification of skin cancer in the initial stages is significant because primary tumours can be removed by surgical interventions, local applications, or cryotherapy while the late-stage melanoma requires treatment that acts on the whole organism. In immunotherapy with newer drugs acting on immune checkpoints including PD-1 and CTLA-4 and targeted therapy of BRAF mutation in melanomas, the prognosis of patients with the advanced phase has improved. Dam et al. [113] used ultra-small hollow silica nanocarriers to deliver photosensitizer zinc phthalocyanine to squamous cell carcinoma regions by conjugating them with folic acid, which targets folic acid receptors highly expressed in cancer tissues. Mello et al. [114] used phthalocyanine aluminum chloride (AlPc) encapsulated in butter-based solid lipid nanoparticles and showed enhanced permeation through the skin and increased delivery to tumor sites. It also aided in the generation of ROS, increased expression of caspase-3 (involved in apoptosis), and decreased expression of Bcl-2 (an anti-apoptotic protein) at the tumor site, indicating selective tumor cell toxicity. Cao et al. [115] used ginseng-derived nanoparticles therapy to suppress melanoma growth by increasing the presence of M1 macrophages in tumor tissue. Liu et al. [116] used chitosan-poly(acrylic acid) nanoparticles loaded with R848 and $MnCl_2$ that promoted the M1 phenotype in macrophages and inhibited melanoma cell proliferation. Xue et al. [117] synthesized silica core nanoparticles with etched polydopamine and loaded it with JQ-1, a PD-L1 inhibitor, exhibited sustained drug release, reducing PD-L1 expression on melanoma cells. Kuang et al. [118] used Ag nanoparticles coated with sucrose which promoted their tumor-infiltrating CD8+ T cells and augmenting the effectiveness of anti-PD-1 treatment in melanoma. Suarasan et al. [119] tested and confirmed the Au nanoparticles efficiently absorb photon energy and convert it to heat upon laser exposure, leading to damage to nearby cancer cells, making them effective in PTT in melanoma tumor model. Jeon et al. [120] used Au nanoparticles conjugated to anti-HER2 and melanoma-associated antigen antibodies which enabled selective killing. Himalini et al. [121] synthesized Ag nanoparticles from *Fusarium incarnatum* fungal extracts which inhibited the tyrosinase activity, maximizing their cytotoxic effects in human melanoma SK-MEL-3 cells. Md et al. [122] synthesized α-mangostin-loaded particles topical gel formulation that exhibited penetration up to 230.02 μm.

4.6 Leukaemia

Leukemia is categorized as a hematologic cancer, a disease that affects the blood and starts in the bone marrow creating an unusual growth of astounding and uncontrolled white blood cells. It is broadly classified into four major types viz. acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). Acute leukemias are high-grade types that evolve rapidly and demand aggressive therapy, whereas chronic leukemias are lowgrade diseases that may remain clinically silent for years. These include for example chromosomal translocations and translocations that are characteristic of CML and the Philadelphia chromosome. Leukemia, unlike other cancers, has no single treatment approach due to the several types that exist, but it easily responds to chemotherapy, targeted therapies, radiation and when all fails, hematopoietic stem cell transplantation is considered. Novel agents for CML, such as tyrosine kinase inhibitor based therapies and for CLL, monoclonal antibodies have proven to enhance the prognosis of certain patient groups. Absolute results still remain poor for acute leukemias particularly in patients and elderly persons. Current efforts to improve treatment are directed mainly in immune therapies like Chimeric antigen receptor T-cell therapy that has some efficacy in relapse/ refractory setting. While new approaches to diagnostics and treatment permitting early diagnoses yield higher survival rates, problems associated with resistance to therapies and long-term effects of therapies persist. In a very interesting study [123], synthesized thiolated Sgc8c aptamer immobilized on Au nanoparticles coated with magnetic Fe_3O_4 NPs forming a aptasensors hairpin structure and incorporated with ethidium bromide for the quantification. The hairpin structure is disrupted when aptasensor is sensitized with leukemia cells. Grechkin et al. [124] used Sgc8 aptamer conjugated with amino- and carboxyl-modified silica-coated terbium (III) thiacalix arenesulfonate to form luminescent nanoparticles that exhibited a larger shift in fluorescence intensity in human T lymphoblast CCRF–CEM cells compared to Jurkat cells due to differences in the concentration of tyrosine protein kinase 7 on the cellular membrane. Haghighi et al. [125] used fluorescent Au nanoclusters combined with superparamagnetic (Fe_3O_4/GNCs) nanoprobe and shown the ability to uptake and image HL-60 cancer cells. Bordbar et al. [126] synthesized paper-based sensor array containing 16 nanoparticles deposited on a sheet of hydrophobic paper in a 4 × 4 array format employing volatile organic compounds (VOCs) in blood samples for detection of leukemia by comparing images of sensors recorded by a scanner before and after exposure to blood vapor with 96% accuracy rate. Wang et al. [127] reviewed the use of iron oxide which serve as contrast agents in MRI, magnetic biosensors, and targeted administration of anti-leukemia medications. Li et al. [128] synthesized mesoporous silica nanoparticles coated with folic acid PEG–lipid bilayer membrane loaded with PTX and tanshinone which targeted folate receptors and facilitated uptake. Shahriari et al. [129] synthesized MWCNT coated with Ag nanoparticles where it exhibited high efficacy against acute myeloid leukemia and acute T-cell leukemia cell lines and increased cytotoxicity. Esmaeili et al. [130] used zinc oxide quantum dot nanoparticles which induced G1 cell cycle arrest mediated by p21, a protein involved in cell cycle regulation and halted uncontrolled growth of NB4 cells. Fan et al. [131] developed double oligopeptide-coupled nanoparticles loaded with arsenic trioxide to target both osteoblastic and vascular niches within the bone marrow which eliminated primitive chronic myelogenous leukemia cells and reduced the size of K562- human umbilical vein endothelial cells organoids.

5 Targeting Cancer Stem Cells

Cancer stem cells are a small population of cells within tumors which have capabilities to regenerate tumor after its eradication. They exhibit very slow proliferation rate but unlimited self-renewal and differentiation capability. They also demonstrate drug resistance, apoptosis resistance and highly efficient DNA repair mechanisms. Along with this, they can also transdifferentiate thereby facilitating epithelial to mesenchymal and mesenchymal to epithelial transition. Owing to their effective tumor initiating capability, they have been widely studied for targeted drug delivery and treatment using nanotechnology [132, 133]. Gold and polymeric nanoparticles have been widely used to target CSCs; a summary of recent studies are represented in Table 8. Achieving the goal of specifically targeting and eradicating CSCs as the latest generation of nanoparticles with rich and diverse capabilities is the top priority. This may be done by considering some significant factors like blood flow, presence of CSCs in the tumour, release of nanoparticles, specific targeting signals, endosomal escape and delivery to organelles such as nucleus. For efficient blood circulation, nanoparticles should try to escape the mononuclear phagocyte system that will decrease their withdrawal from the blood circulation thus extending their stay within the human body. This objective can be attained by using surface-modifying agents, e.g. polyacrylic acid, PEG, dextran, etc. These non-adhesive polymeric moieties are water-soluble and enhance nanoparticle stability. At the same point this improves their circulation and lowers unwanted absorption. Other

Table 8 Polymeric nanoparticles for targeted drug delivery to cancer stem cells

Nanoparticle	Study/ reference	Drug(s)	Targeted biomarker	Results
Poly lactic-*co*-glycolic acid	[195]	PTX, curcumin	Breast tumor cells	Selective targeting of CSCs, inhibition of tumor growth
	[196]	PTX	Folic acid	Reduction in chemo-resistant gene expression, increased apoptotic markers
	[197]	PTX, Salinomycin	Hyaluronic acid	Cytotoxicity against bulk tumor cells and CSCs
Poly lactic-*co*-glycolic acid -PEG copolymer	[198]	All-trans retinoic acid	CD44, CD133	Inhibition of CSC growth
PEG monomethyl ether	[199]	PTX, Survivin siRNA	CD133	Suppression of tumor growth, reduction of tumor size
Hyaluronic acid	[200]	Docetaxel, Photosensitizer	CD44	Growth inhibition of breast cancer CSCs
Liposomes	[201]	Salinomycin, DOX	CD133, EpCAM	Synergistic inhibition of CSC growth

noteworthy stealth features can be derived by wrapping nanoparticles with platelet membranes or hiding the same nanoparticle within self-peptides like CD47. Another parameter is the size of nanoparticles, smaller size decreases circulation time of the particles while larger size limits their penetration inside solid tumors. Nanoparticles also face a tough job to find a compromise between the size dependent and the property that accumulates when the size is larger, while the property's penetration capacity, on the other hand, is becoming more unfavorable when the size is smaller. Nanoparticles having diameters which range from 10 to 200 nm for entry into the blood vessels and then penetration into the tumors. Multiple-stage drug delivery systems like sequential initiative drug delivery are capable of sensing tumor invasion and separate the drug release in segments.

Nanoparticles surface ligands help to latch on CSCs receptors or molecules. Thus therapies get directed to CSCs thereby lowering toxicity and preventing uptake by cells. Antibodies, ligands, and aptamers specific for tumor stem cell surface markers play an important role in nanoparticle delivery. A number of molecules that are therapeutic, require access to some of the cellular locations, including mitochondria, nuclei and other different organelles, in order to work properly. For example, nanoparticle designs with positive charges helps more in penetrating lysosomes due to their ease of entry into these organelles. In addition to therapeutic agent delivery, it is also crucial to deliver diagnostic molecules for imaging of CSCs to track their existence and condition. Quantum dots and photon upconversion nanoparticles among others are vital components in multimodal imaging technique allowing for the real-time visualization of CSCs (Table 9).

Table 9 Au nanoparticles in cancer stem cell therapy

Nanomaterial	Description	Application in CSC therapy	References
Au Nanorods	Proven to have great NIR laser-induced PTT efficacy.	Used for molecular imaging and targeting tumor cells. Can kill both cancer cells and CSCs. Aptamers can be linked on the surface of GNRs to target CSCs. Suppresses stemness gene expression, inhibits formation of mammospheres, and reduces CSC subpopulation. Utilized as a carrier for drug delivery to achieve combined PTT and chemotherapy for triple negative breast cancer.	[203]
Au Nanoshells	Consist of a thin gold layer on solid core nanoparticles.	Used as PTT agents for CSC-based cancer treatment. Combined with radiation to eliminate radiotherapy-resistant breast CSCs. Enhanced destruction of tumor cells and decreased CSC subpopulations.	[202]
Au nanoparticles	Biocompatible and applied in various biomedical fields.	Conjugated with salino-mycin for specific targeting and high antitumor efficacy against breast CSCs. Induce Fe-dependent cell death (ferroptosis) and PTT in breast CSCs. Synthesized as a photomedicine for synergistic PTT and photodynamic therapy.	[204, 205]
Au Nanocages	Convert NIR light to heat and exhibit radio-sensitization.	Synthesized as CD44-targeted nanoparticles for synergistic thermotherapy and radiotherapy against CD44 highly expressed cancer cells. Result in increased tumor cell apoptosis and significant tissue necrosis. Few studies on Au nanocage-based PTT for CSCs have been reported, warranting further research to improve safety and efficacy.	[206, 207]

6 Conclusions

By resolving long-standing issues with traditional chemotherapy, the field of advanced nanoparticle-based therapies holds significant promise for changing cancer treatment. The examined body of research highlights the various uses and potency of nanoparticle formulations in delivering therapeutic payloads to tumor locations with the least amount of systemic harm. Surface modifications for targeted delivery, regulated drug release profiles, and the capacity to encapsulate numerous medicines for combination therapy are just a few of the special benefits that come with using nanoparticles. Since liposomes and inorganic nanoparticles are two examples of nanoparticle platforms that are versatile, it is possible to precisely tune their physicochemical features to maximize therapeutic effects. The prospects of nanoparticle-based cancer treatments to improve patient outcomes across a range of cancer types is highlighted by their effectiveness in preclinical investigations and clinical trials. Specifically, formulations including nanoparticles have proven effective in improving the therapeutic index of traditional anticancer medications and surmounting drug resistance processes. Nonetheless, there are still a number of obstacles in the way of clinical translation and broad use of treatments based on nanoparticles. Managing concerns about long-term biocompatibility and safety, assuring reproducibility and scalability of manufacturing processes, and negotiating regulatory paths for approval and commercialization are some of these obstacles. Future research should concentrate on combining nanotechnology with cutting-edge cancer treatment approaches like immunotherapy and customized medicine. Synergistic therapeutic techniques can be established to improve treatment outcomes and increase antitumor immunity by utilizing the unique capabilities of nanoparticles to influence immune responses and deliver immunomodulatory drugs. In summary, this comprehensive review underscores the transformative potential of advanced nanoparticle-based therapeutics in reshaping the landscape of cancer treatment. Continued interdisciplinary efforts involving collaboration among researchers, clinicians, industry partners, and regulatory agencies will be essential to realize the full clinical impact of nanoparticle-based approaches and ultimately improve patient care in the fight against cancer.

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Fabrication of Advanced Nanohybrid Materials and Their Deployment in Electrochemical Sensing of Diverse Analytes

Nandita Singh, Uday Pratap Azad, Ananya Srivastava, Rohini Kumari, Rajendra Prasad, and Pranjal Chandra

1 Introduction

Nanoscale structured materials have a vast and growing societal impact [22, 30, 49, 51] and to solve environmental, energy, and health related issues, their invention has garnered a great deal of interest. Every day, a large variety of nanomaterials are manufactured for an extensive array of applications in various domains such as the textiles industry, electronics, optics [74, 75, 83, 101], medicinal therapies [6, 8, 64], energy storage/production [94, 115], and diagnostics [19, 93, 116]. Owing to their tremendous shape and size dependent activity and high catalytic property, they have been widely utilized in the construction of extremely sensitive and selective sensors with improved analytical parameters (linear dynamic range, limit of detection, sensitivity), enhanced speed, less response time, reduced construction costs, and increased possibility for automation and miniaturization. To develop the sensors with the above-mentioned features, several novel approaches and methodologies have been adopted in the synthesis of nanomaterials and fabrication of biosensors [10, 36, 46]. Nanomaterials alone may show good performance, but their stability and cost make them challenging to commercialize on an industrial scale, so from this perspective, NHMs are a better alternative. The name "nanohybrid materials" originates from the fact that they contain both synthetic organic and inorganic

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components that are covalently or non-covalently joined at the nanoscale. Among the many intriguing new material classes, NHMs are especially promising. Organic components in NHMs provide better stability and sometimes, due to the synergetic effect, improve catalytic activity. Green, eco-friendly, and multifunctional NMHs are the need of the hour, and they can be used in the construction of biosensors and bioanalytical devices. In the present time, more emphasis is given to create NHMs with desired nanostructures in order to maximize the synergy between the constituents of the NHMs and to improve the structural and electrical characteristics of the final NHMs [26, 27, 63]. In order to use the NHMs for desired analytical or bioanalytical purposes, the physicochemical properties of either inorganic or organic components may be manipulated [9, 49, 51, 73]. Several factors, such as size, shape, and functional groups, have been taken into consideration during the synthesis of the NHMs for their improved catalytic performance in a variety of applications, such as electronics, optics, biomedicine, drug delivery, and green fuel technology [5, 74, 75]. During the synthesis of NHMs, advantageous features of organic as well as inorganic components are taken into consideration [32, 50]. The synthesis of NHMs for analytical purposes has advanced and evolved significantly in the last several decades [23, 80]. Over the last decade, the literature on NHMs has increased significantly. Using the PubMed database, an extensive literature search was conducted using the keyword "Nanohybrid Materials for Sensing", and the period 2013–2023 saw the identification of 283 peer-reviewed journal articles (Fig. 1a). All things considered; the literature search reveals a rise in the number of publications during the previous 10 years [5]. As we know, hybridization is the act of combining, integrating, or mixing qualities at the molecular level to produce a hybrid material that has the benefits of many constituents while eliminating the drawbacks of each of individual constituent. By using this procedure, a NHM is produced that has the benefits of many components without the drawbacks of any of those components

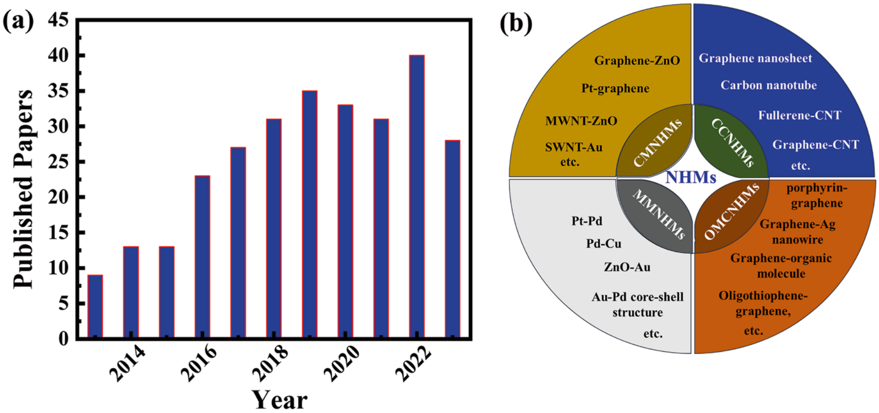

Fig. 1 (**a**) Literature survey for the article published by using the internet database "PubMed" from 2013 to 2023 and by using the keywords "Nanohybrid Materials for Sensing" and (**b**) Classification of various types of nanohybrid materials

[12, 45]. Every substance, even nanoparticles, has unique physical properties depending on its chemical origin. These nanoparticles have different properties when combined, and depending on the synthesis method, one or more of the components may perform better than the others. The changes can be seen in the dimensions, physical appearance, surface chemistry, chemical reactivity, capturing capabilities, energy gap, resistance to decay, electronic and mechanical properties, as well as a few new properties will be generated that will be unique to NHMs. The blending synthesis produces the many forms of core-shell bimetallic NHMs, which include triangular, bipyramidal, rod-like, spherical, cuboidal, plate-like, dumbbell, and polyhedral. Based on the parent materials employed in their production, NHMs are broadly classified into four categories, namely (i) Organo-Metal–Carbon NHMs (OMCNHM), (ii) Carbon–Carbon NHMs (CCNHM), (iii) Metal–Metal NHMs (MMNHM), and (iv) Carbon-Metal NHMs (CMNHM), as shown in Fig. 1b [5]. Hybridization of a large number of carbonaceous nanoparticles would produce CCNHMs, some of which have one or more boundaries, such as carbonaceous nanotubes, fullerenes, or graphene. The name "CMNHMs" refers to the combined components that result from the hybridization of carbonaceous nanoparticles with metals such as gold, silver titania, alumina, and iron oxides. Other types of NHMs are MMNHMs, which can be synthesized by overcoating or a chemical process that blends two or more metal-based NHMs. Sometimes, some biological molecules, such as drugs, proteins, and enzymes, can be attached to metal nanoparticles to create materials that fall under the OMCNHMs category.

These NHMs are essential in many different domains, including biomedical, environmental monitoring and cleanup, heat transfer, paints, temperature sensors, medicinal, and catalysis [4]. The synthesis methods which have been used for the preparation of NHMs can be categorized into two parts: (i) the top-down approach and (ii) the bottom-up approach. In this book chapter, we will try to cover various types of NHMs synthesized via numerous innovative methods which have been used for the construction of a wide range of biosensors. We will also cover the discussion of the developed sensors and their electroanalytical parameters.

1.1 Carbon-Carbon Nanohybrid Materials Based Electrochemical Sensors

This is an important and relatively cheaper NHMs which has been used for various applications. The three main carbon nanostructures which comes under this category and combine to form carbon-based nanohybrids are 0-D fullerenes, 1-D carbon nanotubes (CNTs) (single walled and multiwalled), and 2-D graphene. Cage-like fullerenes and CNTs with open-ended hollow structures offer superior benefits for producing endohedral nanohybrids (NHs) and allowing the formation of their exohedral forms [92]. "Nano-peapods" are fullerenes or graphenes encased within CNTs or CNHs [86] while hybrid fullerene structures with several layers are called "carbon nano-onion" [43, 60, 72]. In contrast, the exohedral conjugation of CNTs, graphene, and fullerenes uses either short-ranged specific contacts or long-range electrostatic interactions. Qu et al. [76], wherein the ensemble process is driven by covalent functions and conjugating molecules or polymers [114]. Some examples of these functionalizations are attaching polar carboxyl or hydroxyl surface groups (-COOH or -OH) to graphene and CNTs via oxidation [15, 76] and the adherence of polymeric assemblies [106] or chemically active compounds [20]. A catalytic reaction is usually used to produce seamless exohedral bonding between CNTs and graphene [114] or CNT and fullerene [70] (nanobuds) through covalent modification. Furthermore, NH-based thin film layered assemblies can be produced by electrostatic and non-covalent interactions using the drop-cast [37], spin-cast [96], and dipping processes [106] of these graphitic NHMs. Electrochemical detection of explosives can benefit from materials with fast charge transfer and excellent adsorptive properties [33]. Graphene is known for its high conductivity, huge surface-to-volume ratio, and chemical stability [53, 111, 112] Therefore, it is frequently utilised as an electrode modifier [59, 90] Nevertheless, due to van der Waals interactions between the individual graphene sheets, it has a tendency to restack, which will decrease the specific surface area that is available and restrict its applied applicability [39, 65]. A popular strategy to get over the restriction is to combine graphene with other nanomaterials. The insertion of CNTs has been shown in earlier research to significantly reduce graphene nanosheet re-coating, hence boosting the contact surface area of electrolytes [24]. CNT-based chemical analysis and research in a variety of industries, such as the chemical, food, pharmaceutical, and environmental sectors, heavily relies on electrochemical sensors [35, 84, 108]. It can be easily adjusted with different nanoparticles due to their structural chain, which makes them appropriate for creating nanocomposite films. Because of the excellent mechanical, physicochemical, and electrical properties of CNTs, nanocomposites made of them are frequently used in the creation of sensors. Also, even at low analyte concentrations, they exhibit high detection sensitivity [67]. The strong hydrophobicity of CNTs makes them insoluble and leads to their aggregation in water, which is the primary barrier to their use. Another important concern is the intrinsic cytotoxic properties of CNTs [48, 66]. But by modifying CNTs, some of these limitations might be lifted. The ability to chemically tune and modify the surface of CNTs is one of the biggest advantages of using them in research. Through functionalization, chemical functional groups on CNTs may mitigate their degree of hydrophobicity and toxicity [48, 66]. It is not possible to cover all the sensors based on carbon-carbon NHMs, as there are a large number of carbon-carbon based NHMs, and at the same time, they have been used in the construction of sensors for numerous analytes. However, we would like to cover some important as well as relevant literature in this section, which has been discussed below.

Graphene Oxide-CNT NHMs Based Sensor

This hybrid class of nanomaterials has been used in various types of sensors, and some of the most important sensors will be discussed. Nie's groups proposed a new type of GO-MWCNT nanohybrid material coated glassy carbon (GC) electrode for the detection of Azithromycin (Azi), a commonly used macrolide antibiotic. Here graphene oxide (GO) nanosheets have been used as a surfactant to create a stable MWCNT aqueous dispersion, which is shown in Fig. 2a. Using the ideal conditions, the fabricated sensor showed linear responses over the concentration range between 0.1 to 10 μM of Azi, and the limit of detection was reported to be 0.07 μM [109]. In another study by Lei and his co-workers, an ErGO/MWCNTs NHMs modified GC electrode was developed for the determination of 2,4-dinitroanisole (DNAN). The stepwise construction of sensing probe is depicted in Fig. 2b. They have constructed ErGO/MWCNTs/GCE sensors through the electrodeposition method, which exhibited good reproducibility and high selectivity with two linear responses in the concentration ranges of 0.1–5.0 μM and 5.0–30.0 μM, respectively, whereas the limit of detection was found to be 0.084 μM [24]. A new electrochemical sensor to monitor 5-hydroxytryptamine receptor agonist Eletriptan (ELE) was constructed by Kaya et al. [47]. They have prepared the sensing probe by using GO/Pt-Ir NHMs, and to further increase its sensitivity, they have functionalized it with carboxylic acid and amine functionalized CNTs. They observed the best peak current response of ELE at GO/Pt-Ir/MWCNT-COOH with a linear concentration range of 1×10^{-7} to 4×10^{-6} M and a detection limit of 6.1×10^{-9} M. In another report, Adeniyi et al. [3] developed a non-enzymatic sensor of glucose with sensitivity value of 992.4 μA mM^{-1} cm^{-2} in human saliva. They fabricated it through modification of the GC electrode by SWCNT/rGO/cobalt phthalocyanine NHMs.

Here, the conducting GO and CNT are used to increase the metal phthalocyanine's (MPc's) electrical conductivity. The as prepared GCE-SWCNT/rGO/CoPc electrode shows a linear response for glucose concentration over the range of 0.30–0.50 mM and 0.50–5.0 mM with a limit of detection of 0.12 μM.

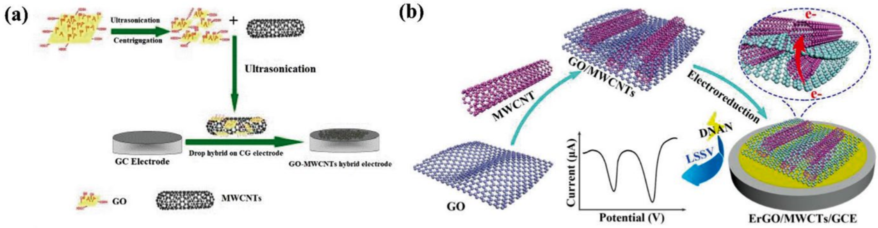

Fig. 2 (**a**) Stepwise fabrication of a sensing probe by using GO and MWCNTs for the detection of Azithromycin. (Adopted with permission [109]) and (**b**) Stepwise fabrication of a sensor and techniques involved in the determination of 2,4-dinitroanisole. (Adopted with permission [24])

Graphene Oxide NHMs Based Sensor

Graphene is a well-known carbon-based material and has been used for the development of various potentially important biosensors. For example, Wong and colleagues developed a novel 4-nitrochlorobenzene (4-NCB, which is one toxic organic pollutant)-based sensor employing carbon nanohorns (CNHs) and GO NHMs (CNHs/GO), which exhibits a linear calibration range of 0.1–90.0 μM with a detection limit of 10.0 nM. Here, the rapid electron transport is provided by the preserved CNHs, and the GO sheets created by partly opening the CNHs offer a greater surface area, a large number of active sites, and increased dispersibility of the CNHs/GO [113]. In another work, Aminabhavi and his group reported a novel GO and g-C_3N_4 NHMs modified GC electrode surface (GO/g-C_3N_4/GCE) for selective detection of methyl parathion (MP) and carbendazim (CBZ) as pesticides used to protect crops and combat pests, which has increased pollution concerns. Under the optimal conditions, they observed a linear response over the concentration range of 8.0×10^{-8} to 1.0×10^{-4} M with a detection limit of 0.824 nM for MP and 1.0×10^{-8} to 2.5×10^{-4} M with a detection limit of 2.82 nM for CBZ [42]. By using the increased electron transport capacity and electrochemical active surface area provided by the assembly of CNHs and rGO, Wu and groups [56] prepared an Au@Pt/CNHs@RGO modified GC electrode for the selective sensing of CBZ in fruit as well as in vegetable juice. The proposed electrode exhibits a linear current response for the oxidation of CBZ between 0.05 and 50 μM with a 1.64 nM limit of detection.

CNT NHMs Based Sensor

CNTs are well-known carbon-based materials which have been extensively employed as electrode modifying materials, and they have also been utilized for the fabrication of various biosensors. A novel electrochemical sensing platform was designed by Zhang et al. [110–112] for the sensing of a cancer biomarker, H_2O_2, released from live cancer cells. The constructed microelectrode sensor was based on carbon fiber covered by Au NPs adorned with nitrogen-doped CNT arrays (CF@NCNTAs-AuNPs which shows a limit of detection of 50 nM and a sensitivity of 142 μA cm^{-2} mM^{-1}. In another research, a binary nanohybrid based sensor was developed by Wang et al. [95] for nitenpyram (NIT) sensing, which is a common nicotinamide pesticide that is chemically harmful. They prepared the sensor by modifying the GC electrode via hydroxylated MWCNT/SWCNT (HCNT) and carbon nanohorns (HCNT/CNH). Under optimized conditions, the fabricated electrode displayed a peak current linear to the concentration of NIT in the concentration range of 20–2000 nM, with a limit of detection of 4.0 nM.

Fullerene NHMs Based Sensor

Fullerene is also a well-known carbon-based material, but as compared to CNTs and graphene, it has been less explored for biosensing applications. In this section, we have tried to cover some important sensors which have been constructed using fullerene as an electrode modifier. For example, a highly-sensitive and quite selective thrombin sensor with an aptamer-recognition surface was proposed by Jamei et al. [44] through the modification of screen-printed carbon electrodes (SPCE) with fullerene (C60), MWCNTs, polyethylenimine (PEI), and polymer quantum dots (PQdot) and tested in human blood serum samples. The steps involved in the construction of the biosensor for the sensing of thrombin are shown in Fig. 3a. In the C_{60}/MWCNTs-PEI/PQdot NHMs, many amine groups provided additional sites for better covalent immobilization of amino-linked aptamer (APT), which increased the aptamer's sensitivity and stability and showed a linear response range from 50 fM to 20 nM, with a limit of detection of 6 fM toward thrombin. In 2022, Wong et al. [102] presented a voltammetric sensor based on hierarchical porous carbon-silver sulphite nanoparticles and employed the differential pulse voltammetry technique for nitrite ion detection in food and river water samples. The sensing probe fabrication and application of the fabricated biosensor for the quantification of nitrite are depicted in Fig. 3b.

They prepared the sensor by modifying the GC electrode via hierarchical porous carbon (HPC) with Ag_2S NPs, Nafion, and C_{60}, where the electrode's increased specific surface area and enhanced electrochemical performance were made possible by the employment of HPC and C_{60}. The as fabricated C_{60}-HPC-Ag_2SNP-Nafion/GC sensor response increases linearly with concentrations of nitrite from 4.0 to

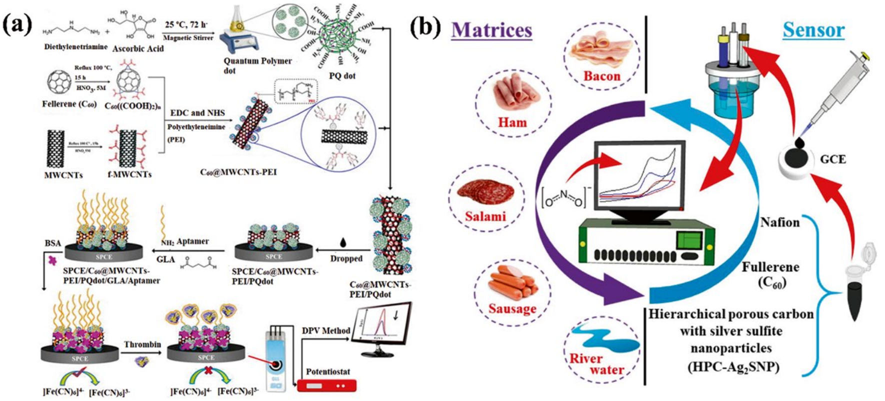
(a)
Diethylenetriamine
Ascorbic Acid
25 °C, 72 h
Magnetic Stirrer
Quantum Polymer dot
PQ dot
Reflux 100 °C, 15 h
HNO3, 5M
Fellerene (C60)
C60((COOH)2)n
EDC and NHS
Polyethyleneimine (PEI)
MWCNTs
f-MWCNTs
C60@MWCNTs-PEI
BSA
NH2 Aptamer
GLA
SPCE
SPCE/C60@MWCNTs-PEI/PQdot/GLA/Aptamer
SPCE/C60@MWCNTs-PEI/PQdot
Dropped
C60@MWCNTs-PEI/PQdot
Thrombin
DPV Method
Potentiostat
]Fe(CN)6]4- [Fe(CN)6]3-
]Fe(CN)6]4- [Fe(CN)6]3-
(b)
Matrices
Bacon
Ham
Salami
Sausage
River water
Sensor
GCE
Nafion
Fullerene (C60)
Hierarchical porous carbon with silver sulfite nanoparticles (HPC-Ag2SNP)

Fig. 3 (**a**) Preparation of PQdots and its integration with MWCNTs and fullerene for the stepwise fabrication of a sensing probe to determine thrombin. (Adopted with permission [44]) and (**b**) schematics for the stepwise construction of a sensing probe comprised of hierarchical porous carbon-silver sulfite nanoparticles and its utilization to determine nitrite in various real samples. (Adopted with permission [102])

148 μM, with detection limits and sensitivity values of 0.09 μM and 0.05 μA μM^{-1} cm^{-2} respectively.

1.2 Carbon-Metal NHMs Based Electrochemical Sensors

In this class of carbon-metal-based nanohybrid materials (CMNHMs), the synthesis involves combining numerous metallic or metal oxide nanomaterials with carbon nanomaterials (CNTs, graphenes, and fullerenes) [103]. CMNHMs are made up of assemblies with various metallic nanomaterials (MNMs), including ligand-based metallic compounds (ferrocene), semiconducting quantum dots (CdSe, CdTe, etc.), metal oxide nanoparticles (ZnO, TiO_2, SiO_2, Fe_3O_4, CuO), and noble metals (Ag, Au, Pt, Pd, Ru, Rh, etc.). Four important mechanisms can be used to synthesize CMNHMs.—(i) using metal nanomaterials (MNMs) to fill the internal spaces of fullerenes and CNTs [98] (ii) MNM attachment to CNT surfaces [18] (iii) adorning carbon nanomaterial (CNM) surfaces with MNMs [104, 105] (iv) In situ development of MNMs on CNM surfaces [117]. Combining graphitic and metallic nanostructures results in novel and advantageous mechanical, electrical, optical, catalytic, sensing, and magnetic properties that may be used in a number of ways. CMNHMs are being used to create better sensors by taking advantage of their increased electrical sensitivity and sorption. The broad range of uses for CMNHMs has sparked extensive study into the creation of new materials and their use in several industries [5]. Because graphene possesses a large specific surface area, strong thermal stability, and outstanding electrical conductivity, it has been widely used in the fields of biosensing [97]. It is also a useful substrate for noble metal nanoparticle support. However, for electroanalysis, Au and Pt NPs with improved catalytic performance have been employed [85]. The use of Pt nanoparticles in biosensor applications was significant. For the nonenzymatic detection of glucose, Pt and Pt-derived alloy electrocatalysts were frequently employed [79, 82, 87]. The dispersal of NPs onto high surface area carbon-derived supporting materials, for example, highly ordered mesoporous carbon [88], carbon nanofiber [91], carbon nanotube [7, 99], etc., has been the focus of efforts to increase the catalytic activity of Pt [31]. Similarly, molybdenum disulfide (MoS_2), a common 2D layered transition metal dichalcogenides made up of stacking S-Mo-S layers through van der Waals interactions, has garnered significant attention in the field of sensing applications due to its profusion in the earth and significant catalytic activity [2, 58]. However, the limited number of active sites and low conductivity of pure MoS_2 limit its potential uses. This basic problem can be resolved by phase engineering metallic 1 T phase MoS_2 or by adorning the MoS_2 with highly conducting materials [62]. On the other hand, it has been demonstrated that decorating with conducting materials is an effective method to improve conductivity and other catalytic capabilities like selectivity, activity, and stability [14, 40, 107].

Graphene-MoS_2 NHMs Based Sensor

Graphene and MoS_2-based NHMs are one category of carbon-metal NHMs and have also been used in the development of various sensing devices. For example, an ultra-sensitive and selective dopamine (DA) biosensor was constructed [69] based on graphene- MoS_2 nanocomposite (pGr-MoS_2), where pulverized graphite (pGr) was used as a precursor of graphene. The sensor displayed a high sensitivity towards DA and showed a linear response with concentrations ranging from 10 pM to 10 μM and a limit of detection of 0.01 nM. Recently, an epinephrine sensor was reported [52] by modifying the surface of the GC electrode with MoS_2 and nitrogen doped rGO (MoS_2/N-rGO/GCE). The proposed sensor exhibits high sensitivity when similar chemicals or prescription medications are also present and has a limit of detection of 3.9 μM and a linear response from 2 to 63 μM. Similarly, Singh and groups [78] reported hydrothermally synthesized MoS_2 and rGO-modified electrodes for the electrochemical quantification of Hg^{2+} ions, where the combined effect of MoS_2 and rGO is responsible for the improved sensing capability. Furthermore, the MoS_2-rGO electrode demonstrated an excellent limit of detection (1.6 μM) with a sensitivity and limit of quantification value of 76.302 $\mu A\ \mu M^{-1}\ cm^{-2}$ and 5.4 μM, respectively.

CNT-MoS_2 NHMs Based Sensor

CNT and MoS_2-based NHMs also comes under the category of carbon-metal NHMs. These NHMs have also been used in the construction and development of various sensing devices. Zhang and co-workers [17] proposed an electrochemical sensor to sense Thiabendazole (TBZ) in food resources, which was constructed by modifying the surface of the GC electrode with MWCNT adorned two-dimensional layered MoS_2. It has been shown that combining MWCNTs with MoS_2 improves its catalytic activity for oxidizing TBZ. The prepared sensor showed linearity toward TBZ in the concentration range of 0.2–1200 μM. Similarly, by using the nanocomposite of molybdenum disulfide nanoparticles (MoS_2NPs) with MWCNT (MoS_2NPs-MWCNTs), a quartz crystal microbalance (QCM)-based molecular imprinted sensor was developed by Capar et al. for sensitive detection of Zearalenone (ZEA) which is found in rice samples and has a carcinogenic effect. Under controlled conditions, the peak current response was linear to the concentration of ZEA in the range of 1.0–10.0 ng L^{-1} with the limit of detection 0.30 ng L^{-1} [11]. Recently, a non-enzymatic glucose sensor [28] was reported, which was constructed using CNT/MoS_2/NiNPs nanocomposites. For this purpose, researchers have first hydrothermally synthesized CNT/MoS_2 in a different ratio, then electrodeposited NiNPs over CNT/MoS_2 to construct the sensor. Under controlled experimental conditions, the constructed sensor shows selective and sensitive detection of glucose over the concentration range of 0.05–0.65 mM with a limit of detection of 0.197 μM.

Graphene-Pt NHMs Based Sensor

Graphene and platinum based NHMs also fall under the category of carbon-metal NHMs. These NHMs have also been widely used in the construction and development of various sensing devices. Although the construction cost of graphene and Pt composite materials-based sensors is very high, various reports are available in which such types of materials have been used in the fabrication of detecting platforms. For example, a non-enzymatic sensor for selective and sensitive quantification of glucose has been reported by utilizing platinum nanoflower-supported graphene oxide (PtNFs-GO) nanocomposites as electrode material for the modification of GC electrode surface [104, 105]. The PtNFs-GO/GC electrode shows enhanced electrocatalytic activity towards glucose oxidation. The constructed sensor shows good analytical performance, such as a low detection limit (2 μM) and a broad linear calibration range (2 μM to 20.3 mM). Similarly, Luo and co-workers [31] reported an extremely sensitive and selective non-enzymatic sensor for glucose quantification. They have prepared it by utilizing rGO and ultrasmall Pt nanowire composites (rGOPtNW) as electrode modifiers. The fabricated sensor shows a linear current response with glucose concentrations ranging from 0.032 to 1.89 mM, with a detection limit and sensitivity value of 4.6 μM and 56.11 μA mM^{-1} cm^{-2}, respectively. In another study, an acetaminophen (ACOP) electrochemical sensor was developed by modifying GC electrode using Au@Pt@Au NPs supported GO nanosheets [85]. Under optimized conditions, the Au@Pt@Au/GO/GCE sensors can be applied for the monitoring of ACOP and showed enhanced electrochemical performance, including a broader linear range of 0.15–125.9 μM and a lower detection limit of 0.045 μM. In another study, Lu and groups [81] reported an electrochemical biosensor based on graphene oxide, platinum nanoparticles (PtNPs), and octahedral UiO-67 metal-organic framework (UiO-67 MOF) nanohybrids (GO/UiO-67@PtNPs) for the selective detection of As^{3+} in the real wastewater sample. Here, Pt NPs can help in selective identification of As^{3+} through their electrocatalysis, while GO can accelerate electron transport and increase the number of active sites. The GO/UiO-67@PtNPs based sensor shows a linear correlation in current for As^{3+} from 2.7 to 33.4 nM with a detection limit of 0.48 nM. In another work, a non-enzymatic biosensor [25] was reported for the electrochemical quantification of glucose on GC electrode modified with reduced graphene oxide and cellulose (rGO/CNC) matrix supported Pt NPs, where Pt/rGO/CNC NHMs demonstrated a porous network like structure with well-integrated Pt NPs, rGO, and CNCs. The Pt/rGO/CNC/GCE sensor showed good sensing performance for glucose quantification from 0.005 to 8.5 mM and a limit of detection of 2.1 μM.

CNT-SiO_2 NHMs Based Sensor

CNTs and SiO_2 NHMs also comes under the category of carbon-metal NHMs. These NHMs have also been used in the fabrication and development of various sensing devices. For example, an electrochemical sensor to monitor gallic acid

(GA) was reported by Zhao and groups [34]. The sensing was done for two types of tea samples (green and black tea). They prepared the sensor via modifying GC electrode by employing SiO_2 NPs and SWCNT nanocomposite. Here, SWCNT, which is interconnected with a carbon conductive network, shows a large specific area, effective charge transport channels, and high conductivity. GA's electrochemical determination performance was improved by the combined action of SiO_2 nanoparticles and SWCNT. The non-conductivity of SiO_2 nanoparticles could be significantly mitigated by SWCNT, and silanol and hydroxyl groups present in SiO_2 are beneficial for the enhancement of GA molecules over the surface of the SWCNT@SiO_2/GC electrode. The prepared SWCNT@SiO_2/GCE showed a linear current response for oxidation of GA between 0.03 and 20 μM with a limit of detection of 8.05 nM. 6-thioguanine (6-TG) is an anticancer medicine and is advised to be taken in a limited dose; if taken beyond the limited dose, it has severe adverse effects, so it is highly desirable to develop an effective sensor for the accurate quantification of 6-TG. Recently, a sensitive sensor was reported for the determination of 6-TG [1]. For this purpose, firstly, Fe_3O_4@SiO_2/MWCNT nanocomposite was synthesized and utilized for the modification of SPCE to fabricate the sensing probe. The peak current of the prepared sensor varies with concentration from 0.01 to 280.0 μM, with a detection limit of 1.0 nM. Venlafaxine, often known as VEN, is an antidepressant that falls under the broad category of selective norepinephrine and serotonin reuptake inhibitors. Neiestani's groups [68] proposed SPCE modified Fe_3O_4@SiO_2/MWCNT electrode for the quantification of VEN. The peak current of the developed sensor was found to increase linearly with VEN concentration from 0.5 to 200.0 μM, with a very small limit of detection of 0.3 μM.

1.3 Organo-Metal-Carbon Based Nanohybrid Materials

Under this category of NHMs, proteins, enzymes, and biomolecules are attached to metal nanoparticles. Some examples of NHMs which fall under this class have been shown in Fig. 1b. They have been used in the construction of sensing devices, and some of them have been discussed below.

Graphene and Silver Nanowire Based Biosensors

Graphene is a well-known two-dimensional electrode-modifying material which has been frequently used in electronics, device fabrication, and biosensor construction. It has been used alone, or in combination with some other nanoparticles, or with other redox probes. For example, NHMs of rGO and silver wire NHMs (AgNW/rGO) [55] have been prepared by means of a two-step process which involves first preparing AgNW/GO and then applying the microwave-abetted hydrothermal (MAH) approach (Fig. 4a). The prepared materials were analyzed using microscopic techniques such as X-ray diffraction (XRD), transmission electron

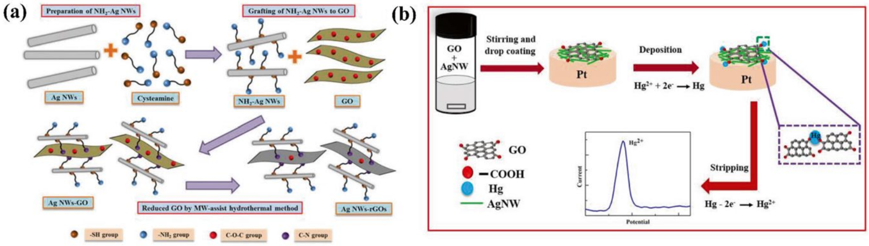
(a)
Preparation of NH_2-Ag NWs
Grafting of NH_2-Ag NWs to GO
Ag NWs
Cysteamine
NH_2-Ag NWs
GO
Ag NWs-GO
Reduced GO by MW-assist hydrothermal method
Ag NWs-rGOs
-SH group
-NH_2 group
C-O-C group
C-N group
(b)
GO + AgNW
Stirring and drop coating
Pt
Deposition
$Hg^{2+} + 2e^- \rightarrow Hg$
Pt
Hg
GO
—COOH
Hg
AgNW
Hg^{2+}
Current
Potential
Stripping
$Hg - 2e^- \rightarrow Hg^{2+}$

Fig. 4 (**a**) Schematics for the preparation of amine-functionalized Ag nanowire and rGO and silver nanowire composites. (Adopted with permission [55]) and (**b**) stepwise fabrication of sensors by using GO and AgNW for the detection of mercury (Hg^{2+}). (Adopted with permission [77])

microscopy (TEM), scanning electron microscopy (SEM), and X-ray photoelectron spectroscopy (XPS). Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) were used to quantify dopamine (DA), ascorbic acid (AA), and uric acid (UA), concurrently over the AgNW/rGO-modified SPCEs. In contrast to pure rGO-modified SPCEs, the AgNW/rGO composites show better catalytic activity, as evidenced by the well-resolved peaks, augmented peak currents, and decrease in overpotential in the concurrent detection of AA, DA, and UA. It was found that the peak current of AA, DA, and UA increases linearly with concentration from 45 to 1550, 40 to 450, and 35 to 300 μM, and the limit of detection was found to be 0.81, 0.26, and 0.30 μM (S/N = 3) for AA, DA, and UA, respectively, at AgNW/rGO-modified SPCEs. It was anticipated that the covalent connections forming between AgNWs and rGOs would inhibit AgNWs from randomly attaching themselves and instead create a porous, continuous network of electrical conductivity that would aid in reactant and electron transport. AgNWs/rGO composites are attractive electrode materials for electrochemical biosensors because of their superior sensitivity. In another paper, an extremely sensitive as well as selective biosensing platform has been reported for the quantification of Hg^{2+} in an aqueous medium [77]. Hg^{2+} has been measured using square-wave anodic stripping voltammetry (SWASV) with a platinum (Pt) electrode, GO, and AgNW nanocomposites (Fig. 4b).

A significant improvement in electron transport rate and Hg^{2+} sensing behaviour is made possible by the combined action of conductive AgNW and GO. Under controlled experimental circumstances, the constructed sensor shows a linear correlation in peak current and concentrations of Hg^{2+} from 1 to 70 nM with a sensitivity of ~0.29 μA/nM. The modified electrode made of GO/AgNW nanocomposites has a detection limit of around 0.1 nM for Hg^{2+}, which is much lower than the WHO safety limit. As compared to other heavy metal ions that interact, such as Pb^{2+}, Cd^{2+}, Cu^{2+}, Na^+, and Ag^+, the sensor reacts to Hg^{2+} very selectively. Its great degree of reproducibility and repeatability is another feature of the sensor. Using tap water samples, the sensor detects Hg^{2+}; its remarkable performance suggests that it is a highly promising platform for on-site Hg^{2+} in water monitoring. Recently, by employing SWASV, a novel method [71] has been developed for the construction of high-performance electrochemical sensors for lead ion detection. The sensor's working electrode was made of GO and AgNWs on polyethylene terephthalate substrates. Investigations were conducted on the electrode's morphology, electrochemical characteristics, and operating factors. A good correlation between current and lead ion concentration (0.2–60 μg L^{-1}) was observed, and the detection limit was found to be 0.067 μg L^{-1}, which is less as compared to the safety standard of the drinking water set by the WHO. By combining 1D Ag nanowires and 2D graphene sheets via basic hydrothermal sol-gel process, an ultra-sensitive platform was constructed. The created Ag-NW/graphene hybrid 3D glucose biosensors [61] showed improved sensitivity of 1249.1 μA mM^{-1} cm^{-2}, as well as a high specific surface area of 119.3 m^2 g^{-1}.

1.4 Metal-Metal Nanohybrid Materials for Biosensing

This is another class of NHMs. Under this category, one metal is attached to another metal at the nanoscale level, which includes ZnO_2-Au, Ag/TiO_2 nanosheets, Pd-Pt, Pd-Cu, etc., as shown in Fig. 1b. This class of NHMs is used in various applications. In this section, we will discuss the sensing applications of some of these NHMs in great detail.

ZnO-Au Nanohybrid Material for Sensing Applications

ZnO-Au comes under the category of metal-metal NHMs. ZnO is a well-known material which has been frequently used with some other metal nanoparticles for the construction of biosensing platforms. Recently, an extremely sensitive detection system [54] ZnO/Au/GaN was constructed for the quantification of alpha-fetoprotein (AFP). High photogenerated current density is promoted by the sensor's fabrication with GaN substrates coated with nanogold sheets, which supply an auxiliary bias voltage. The photocurrent produced by the sensor was much larger when a ZnO/Au/GaN based sensor was employed as a photoanode instead of Au/GaN or ZnO/ITO alone. Antibody modification of the ZnO nanorod arrays was used to allow for the selective detection of AFP. It exhibited an astonishingly low limit of detection of 0.027 ng mL^{-1} (S/N = 3), and the sensor signal response was found to be linear with the AFP concentration from 0.080 to 5.0 ng mL^{-1}. These findings show that this self-powered sensor has the ability to identify the presence of AFP in serum samples. The work offered a new method for creating self-powered sensors that are both extremely sensitive and selective for biomarker detection. They have also reported that this method may help with the early identification and clinical diagnosis of different cancer kinds. In another report, a microstructured elastic polyamide filter membrane (P.F.) substrate was used [13] to prepare thin films of Au and Au/ZnO, which they have characterised using XRD and FESEM techniques. Further, these two surfaces were modified with ferrocene (Fc), methanol (Me), and MWCNT. Finally, these two flexible electrode surfaces were modified with glucose oxidase (GOx). Later on, these two modified surfaces were used as sensing probes for the analytical determination of glucose. The schematic for the electrode fabrication and detection of glucose by using the same is given in Fig. 5a. To check out the influence of ZnO, a comparison of the currents of these two electrodes was made. The electrochemical response of glucose was measured at these two flexible electrodes surfaces, and it was found that both electrodes show a linear response from 0 to 12 mM; however, the use of ZnO thin film resulted in improved sensitivity (30.00 μA mM^{-1} cm^{-2}) and reduced the limit of detection (0.25 mM and 0.83 mM, respectively, for both surfaces). The findings imply that the ZnO film as well as the GOx enzyme work together to boost the biocatalyst's immobilization process, which raises the glucose biosensor's parameters. In another ZnO/Au-based enzymatic biosensor [100], nanocomposites of Au and ZnO (ZnO/Au) were prepared through a simple hydrothermal process. In this process, Au nanocrystals were grown over the surface of ZnO. For the purpose of the morphological investigation, the synthesised ZnO/Au nanocomposites were examined using TEM, SEM, and XRD.

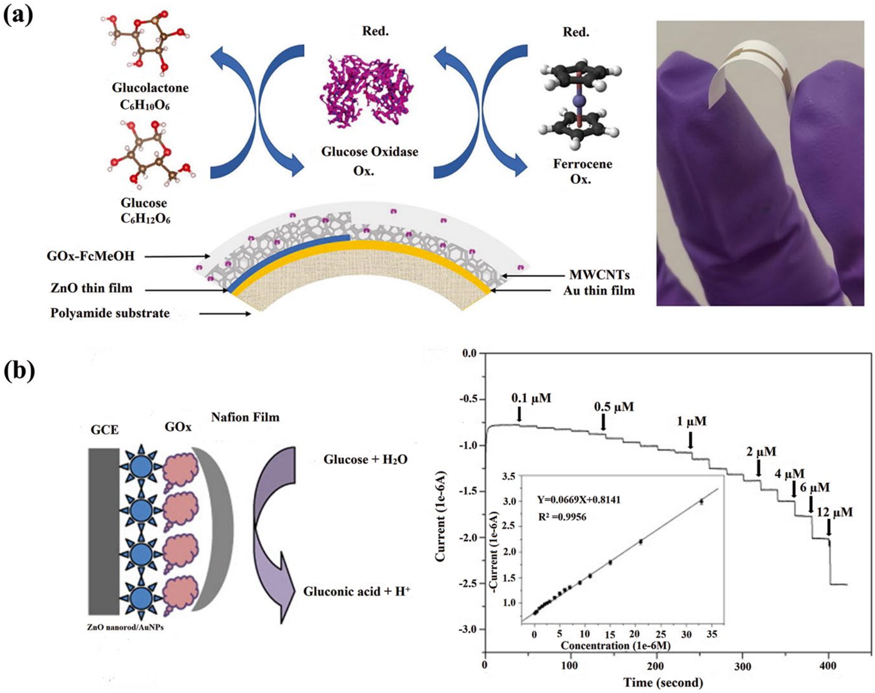
(a)
Glucolactone
$C_6H_{10}O_6$
Glucose
$C_6H_{12}O_6$
Red.
Glucose Oxidase
Ox.
Red.
Ferrocene
Ox.
GOx-FcMeOH
ZnO thin film
Polyamide substrate
MWCNTs
Au thin film
(b)
GCE
GOx
Nafion Film
Glucose + H_2O
Gluconic acid + H^+
ZnO nanorod/AuNPs
0.1 μM
0.5 μM
1 μM
2 μM
4 μM
6 μM
12 μM
Current (1e-6A)
Time (second)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
0
100
200
300
400
Y=0.0669X+0.8141
R² =0.9956
-Current (1e-6A)
Concentration (1e-6M)
3.5
3.0
2.5
2.0
1.5
1.0
0
5
10
15
20
25
30
35

Fig. 5 (**a**) Construction of a biosensor for the quantification of glucose employing MWCNTs, FcMeOH, GOx, ZnO, and Au thin films [13] and (**b**) schematic showing the glucose detection by electrochemical approach utilizing a GC electrode modified by ZnO and Au hybrid material (ZnO/Au); and response of the biosensor with different glucose concentrations in a 0.1 M phosphate buffer solution at pH 7.4, and an applied potential of 0.55 V (vs. Ag/AgCl) (inset shown the corresponding calibration plot). (Adopted with permission [100])

The prepared composites were found to be highly biocompatible, and at the same time, they showed good electron transport properties. The biosensor was constructed by using cross-linking technique (Fig. 5b). The constructed biosensor showed a linear response for glucose detection from 0.1 to 33.0 μM ($R^2 = 0.9956$), and its limit of detection was found to be 10 nM (S/N = 3). The biosensor showed excellent storage stability, a quick reaction time (less than 5 s), high and repeatable sensitivity, and a strong affinity for GOx ($K_M^{app} = 0.41 mM)$. The biosensor's high selectivity was demonstrated by the minimal impacts of electroactive interferents under testing settings. This ZnO/Au combination is thought to be a desirable material for the creation of effective amperometric biosensors.

TiO_2/Ag Nanowire Based NHMs for Sensing Applications

TiO_2 and Ag composites are also an examples of metal-metal nanohybrid materials. A large number of sensing platforms have been constructed using these NHMs. For example, by using simple techniques, a porous film of Ag-TiO_2 which has a structure similar to nanocage (NC) [21] was created and used as a very sensitive, reusable, and affordable SERS substrate. The produced TiO_2 film nanocage structure was first immobilized over a glass plate using the doctor-blade technique. Next, a thermal evaporation approach was used to prepare a thin layer of silver over the surface of the plate with an ideal thickness of 10 nm. The unusual and uneven development of Ag film is caused by the distinct mesoporous structure of TiO_2 film. The formed Ag nanostructure has a spherical shape, is porous, and has an average distance of 10 nm between successive nanostructures (Fig. 6a). With the help of localizing the incident electric field inside the limited volume, these intricately decorated Ag nanostructures create hotspots. The TiO_2 film's distinctive nanocage structure gives the analytes a greater effective surface area for adsorption. Thus, using a tiny portable Raman spectrometer and the probe molecule rhodamine-6 g (R6G), the Ag-TiO_2 NC structure's synergistic effects are amplified by a factor of 10^8. By using a 3D FDTD simulation that closely matched the experimental results, the extent of electromagnetic amplification was further examined. After being exposed to UV light for 130 min, the produced SERS substrate's recyclability was investigated. Under UV light, the dye molecules are sequentially broken down due to the enhanced photocatalytic activity of Ag–TiO_2 NC. Additionally, urea concentrations up to 1 mM, which span the critical range of urea level in blood, may be detected with great sensitivity using the Ag–TiO_2 SERS substrate. Consequently, level free biosensors for routine biomolecule measurement in clinical samples may be employed with the optimized, affordable, and reusable SERS substrate. In other studies, in order to create a SERS biosensor for very sensitive quantification of uracil DNA glycolase (UDG), Ag/TiO_2 nanocomposites were synthesized [41] by using the catalytic hairpin assembly (CHA) strategy (Fig. 6b).

Compared to traditional noble metal materials, this permitted a higher Raman amplification of methylene blue because of the charge transfer (CT) action of TiO_2 and the surface plasmon resonance (SPR) of Ag nanocubes (Ag NCs). To improve

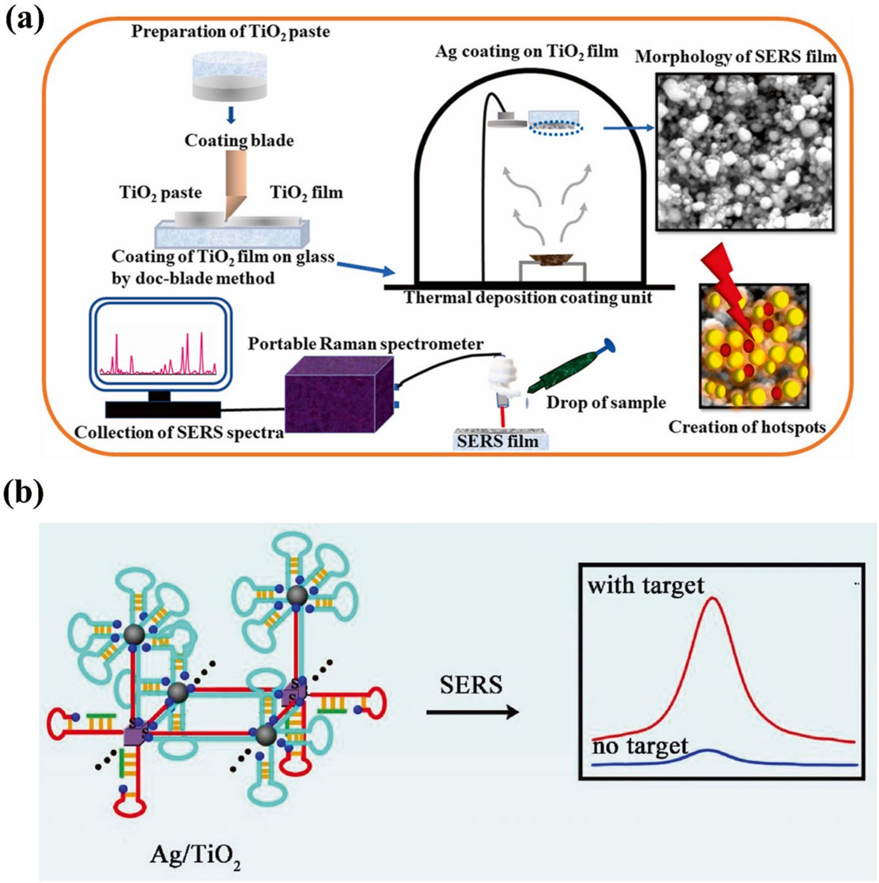
(a)
Preparation of TiO₂ paste
Coating blade
TiO₂ paste
TiO₂ film
Coating of TiO₂ film on glass by doc-blade method
Ag coating on TiO₂ film
Morphology of SERS film
Thermal deposition coating unit
Portable Raman spectrometer
Drop of sample
Creation of hotspots
SERS film
Collection of SERS spectra
(b)
SERS
with target
no target
Ag/TiO₂

Fig. 6 (**a**) Methodical creation of a nanocage-like structure of Ag-TiO_2 as an affordable, recyclable, and sensitive SERS substrate for sensing applications. (Adopted with permission [21]) and (**b**) Fabrication of an Ag/TiO_2 nanohybrid as an innovative SERS substrate for the sensitive identification of uracil DNA glycolase (UDG). (Adopted with permission [41])

the sensitivity of this technology, an impressive way to transform the very little target UDG into various trigger DNA was to incorporate the Pb^{2+} aided DNAzyme cleavage process. Consequently, the SERS biosensor showed outstanding performance with a very low limit of detection of 1.2×10^{-5} U mL^{-1} for the sensitive detection of UDG. A novel approach to designing SERS substrates for biomolecule analysis and illness detection was provided by this suggested experiment. Recently, a unique AgNPs@TiO_2 array-based SERS sensor was reported which has honeycomb-like structure [57] to effectively identify and analyze micro and nanoplastics in ambient water samples. AgNPs@TiO_2 arrays were created by evenly anchoring plasmonic AgNPs within periodic TiO_2 nanocage arrays. Using the Raman spectrometer, the SERS sensor can detect tiny amounts of micro and nanoplastics down to 50 µg mL^{-1} due to the dual augmentation processes in AgNPs@TiO_2. Furthermore, the fingerprint characteristic of this SERS sensor enables it to distinguish between two-component combinations of micro and nanoplastics with success. Moreover, the SERS sensor's high reproducibility (mean standard deviation of 9.69%) ensures the reliability of quantitative detection and enables quantitative measurement of polystyrene (PS) microplastics in soil, lake, tap, and seawater samples with a limit of detection of 100 µg mL^{-1}, 100 µg mL^{-1}, 100 µg mL^{-1}, and 250 µg mL^{-1}, sequentially. With an RSD ranging from 0.49% to 10.23%, the recovery rates of PS microspheres in four different water samples varied in the range of 97.6–109.7%. In another report, by using TiO_2 nanotube arrays (NTAs) and photoreducing Ag nanoparticles after GOx adsorption [29] a GOx/Ag/TiO_2 based glucose sensor was created. Using TEM, SEM, and XRD, the morphology, structure, and element components of Ag/TiO_2 NTAs were examined. TiO_2 NTAs had a homogeneous coating of Ag NPs, with an average grain size of 15 nm. As the NTAs were submerged in the $AgNO_3$ solution for a longer duration of time, the Ag nanoparticle's size and distribution were altered. An opportunity for the identification of micro- and nanoplastic pollutants in ambient water. Ag/TiO_2 NTAs' electrochemical characteristics were assessed using CV and amperometric quantification of H_2O_2. The results showed that TiO_2 NTAs that were immersed for 30 min had the highest electrochemical activity. Under ideal circumstances, the GOx/Ag/TiO_2 NTAs sensor can reach a sensitivity of 0.39 µA mM^{-1} cm^{-2}, with a linear calibration range of 0.1–4 mM.

Pd-Pt Based Nanohybrid Materials Used in Sensing

This is also a class of metal-metal NHMs in which two metal nanoparticles are used together as electrocatalysts; for example, a glucose sensor was created [38] by immobilizing GOx on rGO combined with palladium and platinum NPs (Pt-PdNPs) that were electrochemically formed. In order to potentially use the Pd-Pt alloy in the construction of biosensors, bimetallic NPs were electrochemically formed over the rGO surface. With a wide linear calibration range of 0.5–8 mM and a sensitivity of 814×10^{-6} A/mM cm^2, the as-constructed hybrid sensor demonstrated strong catalytic activity towards H_2O_2. Moreover, GOx-containing active material was incorporated into the rGO/PdPtNPs surface using a straightforward casting technique. They have found that the as constructed biosensor shows good amperometric responses, such as low detection time (5 s), low detection limit, and good sensitivity (24×10^{-6} A/mM cm^2). Furthermore, the impact of interfering materials, repeatability, and sensor stability were also examined.

A highly efficient biosensor for the quantification of glucose was reported by other research group [89], in which they used nanodendrite (ND) of Pd-Pt or Pd-Pt in the construction of the biosensor. By using chitosan (CHIT) as a matrix, GOx was easily immobilized over the surface of the Pt electrode, and later on, GOx was easily cross linked with Pd-Pt NDs with the help of glutaraldehyde. The as constructed biosensor exhibited a wide linear sensing range for glucose quantification from 0.001 to 5 mM and a very low limit of detection (0.6 μM). It was found that the relatively high surface areas, specifically the catalytic activity of Pd-Pt nanodendrites and the outstanding biocompatibility of CHIT, improve the absorption of enzymes and the electron transfer kinetics between the redox enzymes and electrode surfaces, which are responsible for the biosensor's exceptional performance.

To detect miRNA with extreme sensitivity, a novel electrochemical biosensor with triple signal amplification was developed [16]. A bimetallic Pd-Pt supported graphene functionalized screen-printed Au electrode, phosphatase, redox-cycling amplification, and two stem-loop structured DNAs as target capturers were utilized in the construction of the biosensor. With the aid of loop-stem structure probes as well as T4 DNA ligase, the suggested biosensor can distinguish between the target and homologous miRNAs with appropriate selectivity. It was found that its high sensitivity is a result of the improved sensing signal of Pd-Pt supported graphene. Hence, for enhanced quantification and consequent analysis of miRNA in biomedical research and clinical diagnostics, this approach offered a novel and highly sensitive platform.

For the purpose of detecting H_2O_2, a unique, simple, and nonenzymatic electrochemical biosensor platform was created [31]. The modified electrodes of SnO_2/graphene nanosheets (PdPt NCs@SGN/GCE) and Pd-Pt nanocages were used to create the sensor. This method possesses a quick response, good selectivity, exceptional sensitivity, a low limit of detection of 0.3 μM, and a broad linear calibration range from 1 mM to 300 μM. They have found that the PdPt NCs@SGN/GCE demonstrated remarkable electrocatalytic activity towards H_2O_2 It shows promise as a tool for clinical diagnostics to detect H_2O_2 produced from live cells *in situ*.

1.5 Conclusion and Future Prospectives

The progress in the development of smart NHMs is at the forefront of a pioneering discipline which combines material science, biology, and nanotechnology. The hybrid material's already remarkable multifunctionality is enhanced by its nanoscale dimension, structure, shape, and surface chemistry. The synthesis of new nanocomposites and nanohybrid materials has enabled scientists to construct novel electrochemical biosensors with improved analytical parameters. When creating multicomponent systems, it is ideal to preserve the characteristics of the constituent components. When one nanomaterial combines with other materials, contributing nanoparticles may lose a portion of their unique properties. Nanobiosensors have revolutionized scientific research with their improved analytical capabilities. Numerous NHMs have been integrated into the architecture of electrochemical sensors for the quantification of target analytes. NHMs based electrochemical sensors offer superior electroanalytical performance in terms of quick reaction times, affordable transducer surface designs, and long-term stability. Future research endeavours might focus on exploring novel materials, incorporating novel materials to form multi-component systems, and creating controlled and scalable inventive synthesis procedures. Although a great deal of work has been put into this area, many more criteria still need to be considered. The integration of many nanomaterial qualities into a single system has been the subject of research.

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Nanomedicine: Bridging Nanoscience Innovations with Healthcare Solutions

Prem Pandey and Neha Saini

1 Introduction

Bottom can encompass enormous space as quoted by Richard Feynman in his famous lecture “There is plenty of room at the bottom” [1]. When Richard Feynman delivered the iconic lecture back in 1959, no one thought in their wildest dream that nanoscience and nanotechnology will open the pandora box of innovation. The new branch of science unlocked novel opportunities almost in every branch of science (Fig. 1) including medicine, healthcare, agriculture, electronics, environmental engineering, optics, devices, chemical, energy, and automobile [2–6].

To be more precise, nanotechnology engages nanoscale materials and systems typically in the range of 1–100 nm (1 nm = 10^{-9} m). These nanoscale materials and systems demonstrate significantly improved physical, chemical and biological properties. Smaller materials as compared with bulk peers often showcase unique and advantageous properties due to their reduced magnitude. The enormous surface area to volume ratio, diverse shapes & sizes, quantum confinement enables nanomaterials to be customized according to their application [4, 6, 7]. Nano-biosensors, nano-robots, chips with nanomaterials, nano-surface coatings, nano-delivery systems, encapsulating agents, magnetic particles, drug nano-formulations are few examples of this interdisciplinary science where the technology has transcended the boundaries [5, 8, 9].

On the other hand, medicine has its own share of challenges ranging from complex scientific to socio-economic ones. Population explosion, heterogeneity, pandemics, increasing list of new diseases are posing enormous challenges for scientific

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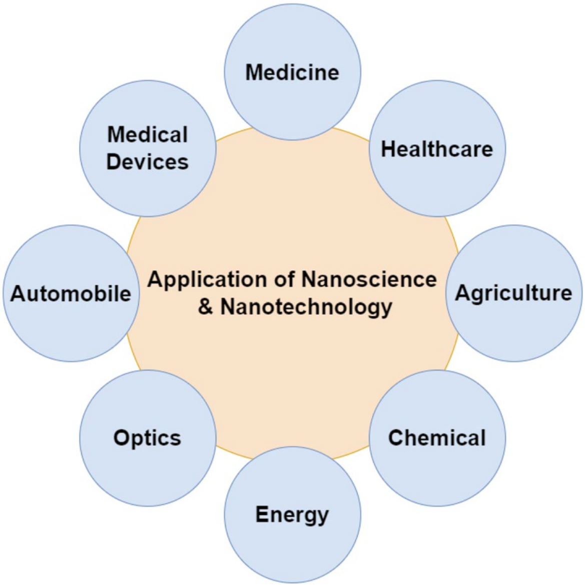

Fig. 1 An illustration of application of Nanoscience & Nanotechnology in different sectors

community for development of precise treatment methods. Recently the world witnessed colossal damage in the humankind history due to worldwide spread of SARS-CoV-2 pandemic in 2020 [10]. It brought world to standstill and caused millions of deaths within short span of time [11]. Researchers across the globe encountered unique problem in reduced vaccine efficacy over a period of time due to emerging variants of SARS-CoV-2 virus [10]. Similarly other diseases like cancer, autoimmunity, Alzheimer's and lifestyle diseases pose challenge in drug development due to non-specificity of the drug, misunderstood drug mechanism, rising costs, and poor pharmacokinetics [12–14].

Amidst these challenges, nanoscience offers a ray of hope. When nanotechnology, nanomaterials and nanosystems are applied to the medicine, it gives rise to new avenue known as nanomedicine [15]. Evolution of nanomedicine has brought significant advancement in cancer therapy [16, 17], tumor imaging [18, 19], disease diagnostics [20], tissue engineering [21] and drug delivery systems [22, 23]. Multiple research reports indicate customised nanomaterials for passive/active targeting, sustained drug release resulting into reduced systemic toxicity and enhanced drug efficacy [22–26]. Numerous nanomedicines have been approved as nano drug delivery systems since approval of DOXIL® (liposomal doxorubicin) in [27–30]. During last few decades more than 100 nanomedicine has been approved by US FDA and around 563 has entered clinical trials [31]. In particular, nanomedicines can provide wide range of treatment options for severe diseases like cancer, autoimmunity and other lifestyle diseases. In this chapter we will discuss the innovation, application and breakthrough for nanomedicine to answer the complex health issues.

2 Nanomaterials

Definition

Nanomaterials can be defined as the materials (≥50%) with at least one dimension in the size range of 1–100 nm as per European commission recommendation 2011. The materials can be natural or man-made. They can be found as aggregate or in free state. Depending upon the application and safety concerns the percentage of materials with specific size range can be modulated [32]. Broadly, nanomaterial can be fundamentally identified on the basis of size & shape, distribution and surface area [32].

2.1 Size & Shape

Size & shape of the material is of prime importance. The material can interact in a unique way with biological systems due to their varied interaction based on different shapes as well as sizes. However, these two properties alone cannot decide the interaction because physicochemical and biological properties of the materials do not change drastically at the size boundary (100 nm) [32, 33]. Also, biological medium greatly influences the nanomaterial's aggregation, stability and surface property in terms of charges [34].

However, the characterisation techniques to determine nanomaterial's size has its own share of limitations in terms of solvent used for synthesis. Ideally, the nanomaterials should be resuspended in the biological medium for the size determination. Sometimes nanomaterials resuspended or incubated in biological medium lead to adsorption of protein corona around nanomaterials [34, 35]. The formation of protein corona around nanomaterials can lead to increase in hydrodynamic diameter, altered physico-chemical as well as biological property and targeting capacity. These nanomaterials can demonstrate different behaviour *in vivo* as compared to *in vitro* systems [36]. Considering the above facts size & shape play important role during nanomaterials' interaction with biological systems.

2.2 Particle Size Distribution (PSD) and Surface Area

PSD speaks about the range of size variation of nanomaterial distribution. Nanomaterials are composed of particles with range of sizes and demonstrate polydispersity. At the same time, if material's surface area by volume ratio is greater 60 m^2/cm^3, it is considered as nanomaterial. However, PSD is given more preference as compared to surface area for determination of nanomaterial identification [32, 33].

2.3 Classification

Nanomaterials can be classified as metallic and non-metallic (Fig. 2). These materials demonstrate therapeutic and imaging properties [16, 17, 26, 37, 38]. They also act as a carrier or delivery agent for antibodies, nucleic acids, clinical lead molecules, biosensors and peptides [22–24, 39–41].

Metallic Nanomaterials

These nanomaterials can be further sub-classified (Fig.2) into pure metallic nanomaterials (e.g., silver, gold, platinum, copper, zinc, titanium, iron, magnesium, and calcium), metal-oxide nanomaterials (e.g., iron oxide, zinc oxide, titanium dioxide and so on), metal sulfide (e.g., copper sulfide, iron sulfide, silver sulfide and so on) and metal organic frameworks (MOFs) [42–44]. Metal nanomaterials has shown a lot of promise in the field of medicine. These nanomaterials can be chemically modified for conjugation with antibodies [45, 46], functionalized with nucleic acids [47, 48], peptides [49], phytochemicals [11, 50] and drugs [51–53]. Numerous reports have suggested their diverse medical applications e.g., drug delivery, enhancement of therapeutic efficacy, antimicrobial activity [11, 47, 49, 53]. The noble metal nanomaterials such as silver, gold, platinum demonstrated excellent anticancer activity [27, 53–56], anti-diabetic activity [52], antimicrobial & anti-biofilm activity [11]. These noble metal nanomaterials have also gained significant attention as an imaging agent and in diagnostic. The noble metal nanomaterials demonstrate amazing surface plasmon resonance properties, high sensitivity, imaging contrast which make them an ideal candidate for imaging [57]. Gold amongst noble metal nanomaterials have been reported to have lower toxicity, enhanced cellular uptake,

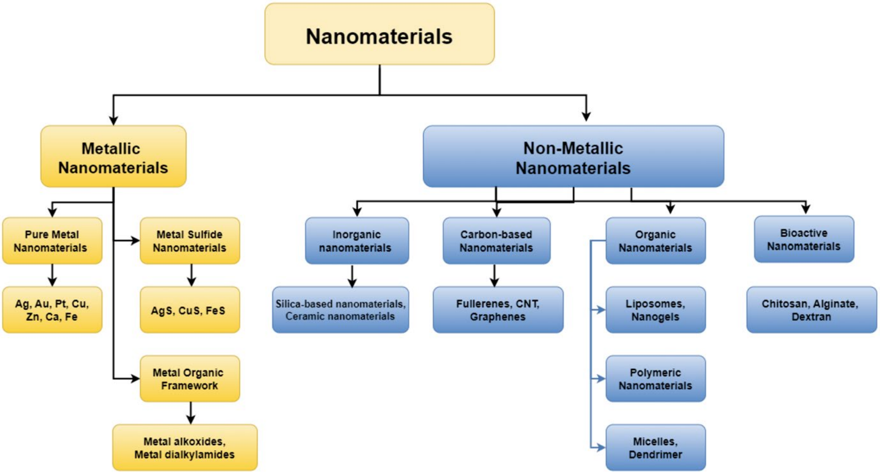
Nanomaterials
Metallic Nanomaterials
Non-Metallic Nanomaterials
Pure Metal Nanomaterials
Metal Sulfide Nanomaterials
Ag, Au, Pt, Cu, Zn, Ca, Fe
AgS, CuS, FeS
Metal Organic Framework
Metal alkoxides, Metal dialkylamides
Inorganic nanomaterials
Carbon-based Nanomaterials
Organic Nanomaterials
Bioactive Nanomaterials
Silica-based nanomaterials, Ceramic nanomaterials
Fullerenes, CNT, Graphenes
Liposomes, Nanogels
Chitosan, Alginate, Dextran
Polymeric Nanomaterials
Micelles, Dendrimer

Fig. 2 Classification of nanomaterials

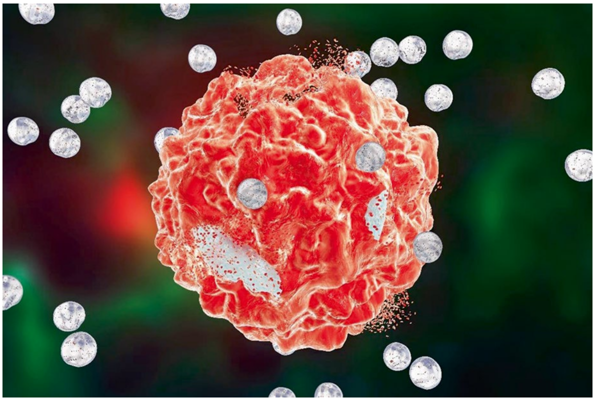

Fig. 3 Representative image for nanomaterial targeted drug delivery. The image has been acquired from adobe stock images (Lic No: AdobeStock_115804667)

biocompatibility, and better pharmacokinetics [57–59]. Whereas platinum is reported to have preferential accumulation at tumor sites, better absorption by tumor cells and causes lysis of DNA strands during cell incubation [60–63]. Also, smaller size nanomaterials demonstrate enhanced permeability and retention (EPR) effect and increased tumor concentrations of contrast agent [58, 59, 64].

During last few decades, metal nanomaterials have been used as drug delivery agent (Fig. 3). Drug delivery systems at nanoscale lead to targeted drug delivery ultimately resulting into reduced side effects and improved efficacy of drugs [44, 65, 66]. The intrinsic characteristics of drug delivery systems such as drug metabolism, pharmacokinetics, half-life and bioavailability can be tuned at nanoscales [22, 24, 44, 66]. Surface modification of metal nanomaterials allows them to bond with targeting agents, biomolecules and drugs [23, 60, 67, 68]. Above properties allow the specific customization of metal nanomaterials and they have been investigated in clinical studies as detection, diagnostic and therapeutic agents [38, 44, 59, 66].

Non-metallic Nanomaterials

The history of non-metallic nanomaterials dates back to as early as 600–300 BC with discovery of carbon nanotubes in pottery from Keeladi, India [69, 70]. Non-metallic nanomaterials include inorganic nanomaterials (e.g., ceramic nanomaterials, silica-based nanomaterials), carbon-based nanomaterials (e.g., Fullerene, Carbon nanotubes, graphene, carbon fibres), organic nanomaterials (e.g., liposomes, nanogels, polymeric nanomaterials, micelle, dendrimer, layered biopolymers), and bioactive nanomaterials (e.g., chitosan, dextran, alginate, hydrogel) [71–75].

Inorganic Nanomaterials

Above mentioned nanomaterials have already been explored for some or other biomedical applications. Bioceramics has been reported for bone, dental and other medical applications e.g., pacemakers, respirators [76]. Silica-based nanomaterials such solid silica and mesoporous silica nanomaterials have been widely used for diverse applications such as contrast agents, drug delivery systems, biosensors, bioimaging, tissue engineering and biomodulation (indirect tumor growth controller) [77–80].

Carbon Based Nanomaterials

Carbon, a multifaceted element has the ability to exist in different dimensions and can be classified into Zero-dimensional (referred as 0-D e.g., quantum dots (QDs), carbon nanoparticles, fullerenes and nanodiamonds), One-dimensional (referred as 1-D e.g., Carbon nanotubes, nanorods, nanofilaments and nanofibers) and Two-dimensional (referred as 2-D e.g., graphene) [9, 81, 82]. This particular ability to exist in different dimensions provide carbon nanomaterials with unique optical, fluorescence properties along with ultra-small size (QDs, 0-D material exist in 2–10 nm range) make them an ideal candidate for drug delivery and as therapeutic agent [82–84]. While another 0-D material, carbon nanoparticles (CNPs) demonstrate biocompatibility, water solubility, small size and improved photostability can serve as good theragnostic molecule [9, 84]. Carbon nanotubes, a one-dimensional (1-D) nanomaterial demonstrated good tumor accumulation *in vivo* and found to be minutely toxic [9, 85]. Graphene, a 2-D carbon nanomaterial have extremely large surface area and is biocompatible. It has been widely used in drug delivery and biomedical imaging [86–89].

Organic Nanomaterials

Organic nanomaterials are usually biocompatible, non-toxic and are biodegradable [90–92]. They offer sustained drug release capacity, have higher half-life, lesser side effects, improved drug efficacy and better patient compliance [92–94]. These materials e.g., liposomes, micelles can be customised for sustained drug release, can target immune cells passively and has better uptake by tissues [95–98]. Liposomes have been widely popular and to date multiple liposomal drugs (>20) have been approved by FDA for diseases such as cancer, inflammatory symptoms and age-related ailments [99, 100].

Bionanomaterials

The biologically active materials having nanoscale precision, organized architecture and surface property are known as bionanomaterials [91, 101]. These materials potentially mimic biological properties of proteins, regulate cellular behaviours, induces biological response *in vivo* and achieve improved therapeutic results as compared with inert biomaterials (Fig. 4) [91, 101–103]. Due to absence of drug loading and drug release systems, these materials achieve even better direct

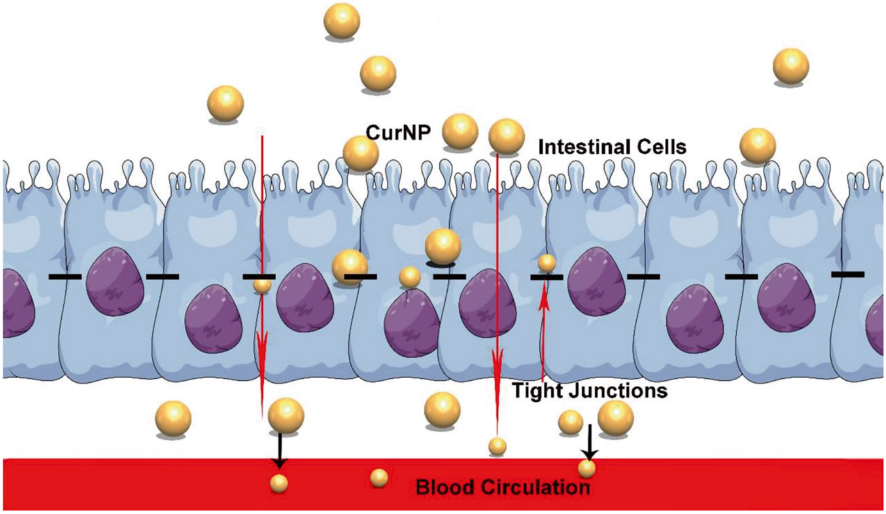

Fig. 4 Representative image for nanodrug uptake by intestinal cells and finally to blood circulation. The original image has been acquired from adobe stock images (AdobeStock_425556323) and necessary modifications have been made

targeting than payload systems and can achieve potentially higher outcome for chronic disease such as nervous ailments and malignant tumors [103]. They halt progression of inflammation by scavenging reactive oxygen species (ROS) [104, 105]. Utilization of modified polyvinyl pyrrolidone (PVP) nanomaterials for removal of ROS in mitochondrial cells and mimicking the antioxidant properties of peroxidases, dismutase and catalases is one such example [106]. PLGA bioactive nanomaterials have been used for anti-tumor activity by polarization of local macrophages [91, 107]. Modified chitosan nanomaterials-initiated tumor-specific immune response and demonstrates anti-tumor effect [91].

Multifunctional Hydrogel loaded with chemotherapeutic drugs has potential application in the photothermal therapy and osteosarcoma [91, 108]. Similarly, modified micelles have been reported in the treatment of Alzheimer's disease [109–111]. Such bioactive nanomaterials show a lot of promise in the field of nanomedicine for treatment of inflammatory diseases, microbial infections, neurodegenerative diseases and autoimmunity [103].

3 Nanomedicine

The global landscape is consistently changing due to emergence of new diseases, socio-economic factors, complexity of healthcare system and challenges in the development of disease diagnostics & therapeutic solutions. The conventional system of disease diagnosis and treatment module are being rendered ineffective due to heterogenous nature of disease and rise in drug resistance [112]. During last century, the progress in biotechnology research contributed significantly towards advancement in next generation medicine development, vaccines, diagnostics and imaging techniques [113].

It has led to the development of personalized and precision medicine [113, 126]. But the high cost of these advanced technologies stays a big concern for healthcare industry [127, 128]. Amidst, these challenges nanoscience and nanotechnology offer a ray of hope as discussed in the Sect. 2 of this chapter.

Nanomedicine, an application of nanotechnology to medicine is based upon three cross functional and progressive molecular technologies: Nanomaterials & nanoscale devices, molecular medicine and molecular machine systems [129, 130]. The living cells, microbes, tumor cells have inherent nanoscale functional components and integration of biotechnology with nanoscience has paved the way to improve disease diagnosis, drug discovery, drug delivery (Fig 3, Table 1) and advancement in pharmaceutical manufacturing methods [21, 26, 103, 113]. Nanomedicine which includes the customised application of nanomaterials, nanodevices, nanotheranostics, nanostructured surfaces and their integration with medical systems will bring much required advancement with cost effectiveness [5, 13, 37, 38, 65]. It has found its way into biocompatible prosthetics, self-organized nanomachine systems (e.g., living cells, folded proteins), nano-biocoatings mimicking natural surfaces, nanovectors for passive as well as active drug delivery systems, nanotheranostics for the disease treatment as well as diagnostics purposes, precision nanomedicine and functionalized nanomaterials for targeted imaging (precise disease investigation) [17, 20, 38, 54, 66, 98]. Nanomedicine has revolutionized the healthcare industry and expanded the market for therapeutic formulations (Table 1).

4 Conclusion and Future Prospects

Significant progress in the field of nanomedicine during last few decades has been clearly evident by the fact that more than 100 nanomedicine has been approved by FDA and EMA [131, 132] and around 500 new nanomedicine candidates (NND) are under different stages of clinical trial [31, 108]. Regulatory agencies have been consistently improving new nanomedicine candidate filing process and promoting their development [131, 133]. Nanomedicine promised a ray of hope for complex diseases such as cancer, autoimmunity, lifestyle diseases and lung diseases. Amongst all liposomal based nanomedicine, nanocrystals and polymeric nanomaterials have been the most successfully commercialized nanomedicine till date. They brought the much-needed revolution in the field of healthcare whether it was therapeutics, diagnostics, imaging, implants or medical devices. However, the rate of NND filing and commercialization is still in its infancy and few concerns are yet to be addressed on a larger scale. A large number of clinical and preclinical studies are required to adequately address the economic viability, safety and efficacy concerns. Governments and industries across the globe are showing higher confidence in

Table 1 List of few FDA approved nanomedicine

Brand name	Nanomaterial	Indication	Year
Adagen	PEG–adenosine deaminase	Severe combined immunodeficiency (SCID)	1990 [114]
Oncaspar	mPEG l-asparaginase enzyme	Acute lymphoblastic leukaemia	1994 [115]
Doxil	PEGylated doxorubicin	Ovarian cancer	1995 [27]
DaunoXome	Liposomal daunorubicin	Kaposi's sarcoma	1996 [116]
DepoCyt	Liposomal cytarabine	Lymphomatous meningitis	1999 [117]
Renagel	Sevelamer arsenite nanomaterial	Chronic kidney disease	2000 [118]
Visudyne	Verteporfin-loaded lipid nanoparticles	Ovarian cancer photodynamic therapy	2000 [119]
Myocet	Liposomal doxorubicin	Metastatic breast cancer	2001 [120]
Somavert	PEG conjugated human growth hormone receptor	Acromegaly	2003 [121]
Abraxane	Albumin nanoformulation of paclitaxel	Breast and pancreatic cancer	2005 [122]
Mifamurtide	Liposomal muramyl dipeptide	Osteosarcoma	2009 [123]
Irinotecan	Liposome formulations of camptothecin	Pancreatic cancer	2015 [124]
BNT162b2	Lipid nanoformulation of mRNA	COVID-19	2021 [125]
mRNA-1273	Lipid nanoformulation of mRNA	COVID-19	2021 [125]

nanoscience research which is evident from higher budget allocation on a consistent basis and rise in nanotechnology-based patent application during last decade. The nanomedicine research domain is riding high on promises and anticipated to make substantial impact in the near future.

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Recent Advances in Nanotechnology for Diagnosis and Treatment of Cancer

Bimal Rajchal, Yub Narayan Thapa, Samrat Paudel,
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1 Introduction

Cancer, a disease featured by uncontrolled and rapid multiplication of cells, is now understood as a condition characterized by the uncontrolled growth of transformed cells that are subject to evolution by natural selection [19]. Programmed cell death (PCD), also known as apoptosis, regulates growth, multiplication, tissue balance, and the natural death of cells in multicellular organisms. Any sort of defect in this phenomenon results in the development of malignancies and, ultimately, cancer [47]. Cancer, with more than 200 varieties, is the second major cause of human mortality, attributing around 20 million fresh cases and approximately 9.7 million deaths (almost 1 in 6 deaths, or nearly 16.8% of total human deaths) worldwide only

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in 2022 [18, 51]. Cancer statistics of 2022 [126] have proclaimed lung cancer to be the most common form of cancer worldwide with 2.5 million novel cases. Breast cancer (2.3 million cases) stands second in the statistics, followed by colorectal cancer (1.9 million cases), prostate cancer (1.5 million cases), and stomach cancer (970 thousand cases) in 2022 [126].

While the majority of cancers develop due to several uncontrollable risk factors, they could be reduced, in many cases, by modifying the day-to-day lifestyle. Regular exercise, a healthy diet, weight management, reduction in alcohol consumption, and smoking cessation are healthy behaviours that humans could adopt to mitigate the threat of cancer [60]. By limiting exposure to chemical carcinogens from cigarette smoke [117], pesticides, and other common household products the beginning stage of cancer can be avoided [13]. The risk of lung cancer can be reduced by quitting smoking and engaging in regular exercise [68]. Similarly, colon, breast, and prostate cancer can be avoided by the reduction in meat [120] and fat [88] in the diet. Nonetheless, a majority of cancers cannot be simply avoided with a lifestyle change but require technology, innovation [46], and medical intervention to cure. One such approach is the implementation of nanotechnology in cancer detection and cure. Cancer nanotechnology combines disciplines of biology, chemistry, material science, and medicine to bring advancement detection, diagnosis, and treatment of cancer [40].

In a broad sense, nanotechnology specifies the design, production, characterization, and use of materials and devices with the smallest functional unit in at least one dimension on the nanometre scale [110]. Generally, a nanometre scale refers to a one-billionth segment of a meter or less than one-tenth of a micrometer in one dimension. A brief yet more informative meaning of nanotechnology would be, “atomically precise technology” or “engineering with atomic precision” [85]. The greater surface charge, minute size, and unique morphology of various polymeric, metallic, and carbon nanoparticles have revolutionized the regime of cancer diagnostics and treatments (Fig. 1). Surface functionalization of these nanoparticles with different biomolecules enhances their properties and makes them suitable for targeted delivery and timely detection of cancer cells [84].

In the last three decades, there has been a notable advancement in the field of nanomedicine, which involves the application of nanoscience and nanotechnology to prevent and treat human diseases. In its simplest form, nanomedicine is the combination of nanobiotechnology and nanopharmaceuticals [24]. Nanobiotechnology reflects the creation, fabrication, and characterization of safe bioactive nanomaterials and devices such as “Quantum Dots” (QDs) and “Cornell Dots” (CU), that can interact with cells and tissues at a molecular level with a high degree of specificity [116], while nanopharmaceuticals refers to nanoparticles such as nanocrystals, liposomes, and metallic nanoparticles meant to transport therapeutic substances to the tissues and cells [15].

Conventional cancer diagnostics methods and cancer treatment methods such as surgery, radiation therapy, and chemotherapy are expensive, time-consuming, and have some side effects. Conventional therapies are aimed to remove diseased cells faster than healthy cells while nanomedicine aims to kill specific cells or repair

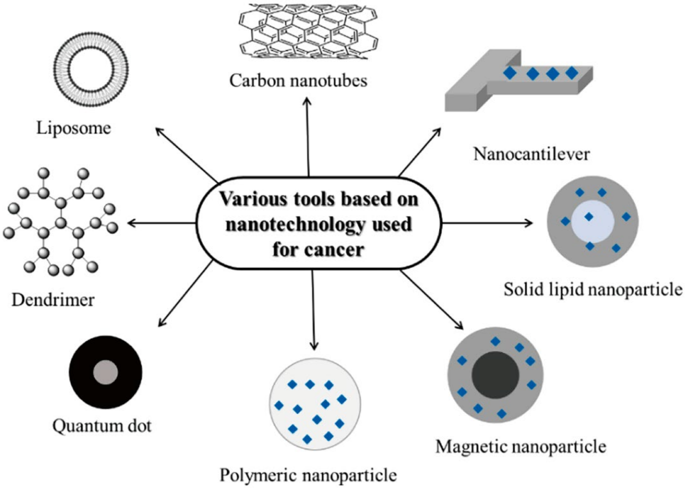

Carbon nanotubes
Liposome
Nanocantilever
Various tools based on nanotechnology used for cancer
Dendrimer
Solid lipid nanoparticle
Quantum dot
Magnetic nanoparticle
Polymeric nanoparticle

Fig. 1 Presentation of various tools based on nanotechnology used for cancer diagnosis and treatment

them individually. This is achieved through the utilization of biosensors capable of detecting and targeting specific cellular abnormalities [105]. By introducing new medicines for both diagnosis and treatment, nanomedicine is bringing out radical changes in clinical practice [22]. It has made it possible to address unmet medical needs by (i) integrating safe molecules that would otherwise be highly toxic, (ii) taking advantage of multiple mechanisms of action, (iii) maximizing efficacy (e.g., by increasing bioavailability) and minimizing dose, (iv) providing controlled drug delivery and site-specific release, favouring a selective distribution and improved transport within the body (e.g., in areas with cancer lesions) and across biological barriers [115].

This book chapter presents an overview of different traditional modalities of diagnosis and cure of cancer and their inherent limitations. Comprehensive illustrations of the recent advancement in the theragnosis of cancer with the leverages of nanomedicine are discussed. Finally, the challenges associated with nanomedicine in combating cancer and the future development of nanotechnology are prospected.

2 Diagnosis of Cancer

The success of the cancer treatment relies on early detection which can improve the survival rate of the patients. This detection can be carried out using various tests such as screening tests, biopsies, blood tests, and diagnostic imaging. Imaging reveals the anatomical, physiological, and functional phenomena dynamically and plays a crucial role in diagnosis and treatment. Cell diseases and cancer were primarily detected in the sixteenth century with the use of a microscope. Furthermore, the development of several diagnostic instruments, such as the X-ray machine, CT scan, and MRI has made diagnosis significantly easier [6].

2.1 Conventional Modalities

There are six traditional imaging modalities to visualize human cancer viz. positron emission tomography (PET), single photon emission computed tomography (SPECT), X-ray computed tomography (CT), optical, Ultrasound (US), and magnetic resonance imaging (MRI). Among these CT, PET, MRI, and SPECT produce three-dimensional (3D) images.

PET is a nuclear imaging method in which a radioactive tracer such as ^{11}C, ^{13}N, ^{15}O, ^{18}F, ^{64}Cu, and ^{124}I along with chelating moieties is injected into a patient's bloodstream. The PET scanner detects the positrons emitted by the tracer as it decays producing 3D images indicating cancerous growth [42]. However, this technology is not very cost-effective. Likewise, SPECT follows a similar principle to PET but with different tracers such as ^{99m}Tc and ^{111}In. SPECT tracers emit single photons which are detected allowing for the visualization of cancerous lesions. It has limited sensitivity and low spatial resolution [55, 81].

Another technique to consider is CT which serves as a precise imaging method that identifies the absorption of X-rays by different tissues as they pass through the body of a subject. However, it has limited compatible contrast agents and has low detection sensitivity. Currently, barium sulphate suspension and water-soluble aromatic iodinated contrast agents are used which have very low retention rates and are not tissue-specific [55].

Optical imaging relies on the properties and interactions of ultraviolet, visible, and infrared light. It can be categorized into bioluminescence imaging which uses native light emitted by the chemiluminescence reaction between enzyme and substrate and fluorescence imaging which is based on absorption of external light by a fluorophore. However, it has challenges of non-quantification and low penetration. Near-infrared (NIR) light provides an effective penetration depth of 7 cm through muscle tissue and 10 cm through breast tissue compared to other wavelengths of the optical range [36]. Combining NIR optical imaging with nanotechnology has the potential to enhance the detection limits and the clinical effectiveness of imaging. Optical imaging offers exceptional detail, while US imaging provides a broader view of live organs. US imaging technique passes high-frequency sound waves of 1–40 MHz into the body of the subject and constructs the images of the scanned areas with the help of reflected waves from the internal organs. It is cost-effective and patient-friendly. However, this modality doesn't assess the whole body and is limited to gas-containing organs like the lungs. The image quality can be significantly enhanced using contrast agents such as gas-containing micro and nanobubbles [99].

MRI is a non-invasive imaging method that provides exceptional anatomical detail and contrast for the soft tissues. However, it is expensive and time-consuming. MRI contrast agents enhance the signal and provide 3D images of high spatial resolution. These contrast agents are paramagnetic complexes such as gadolinium (Gd^{3+}) chelates. Meanwhile, there is a risk of inaccuracies from false-positive contrast enhancement [81, 121].

All these traditional imaging techniques don't give the best results in standalone and these are more complementary rather than competitive. Thus, multimodal imaging techniques, where more than one techniques are combined, result in improved image quality. However, the quality of the image depends on the imaging contrast agents. The utilization of nanomaterials for precise targeting and molecular imaging is a promising way to enhance cancer therapy.

2.2 Nanotechnology-Based Imaging Modalities

Conventional imaging techniques fall short of an accurate diagnosis of human cancer as noticeable morphological changes are absent in the early stage and the resolution of these conventional diagnostic imaging is insufficient [109]. Meanwhile, the tumour cells can evade detection for over a decade following the onset of the tumour and there is a challenge of identifying lethal cancer and minimizing false positive results. Nanoparticles possess optical, magnetic, acoustic, and structural properties that act as contrast agents to enhance imaging capabilities (Fig. 2) [131]. They target the biomarkers that help in the screening, diagnosing, and monitoring of various types of cancers [65]. Thus, nanotechnology enhances the signal strength for increased sensitivity, boosts multiplexing capabilities to enhance specificity, and leverages artificial intelligence for more accurate early diagnosis of lethal cancers

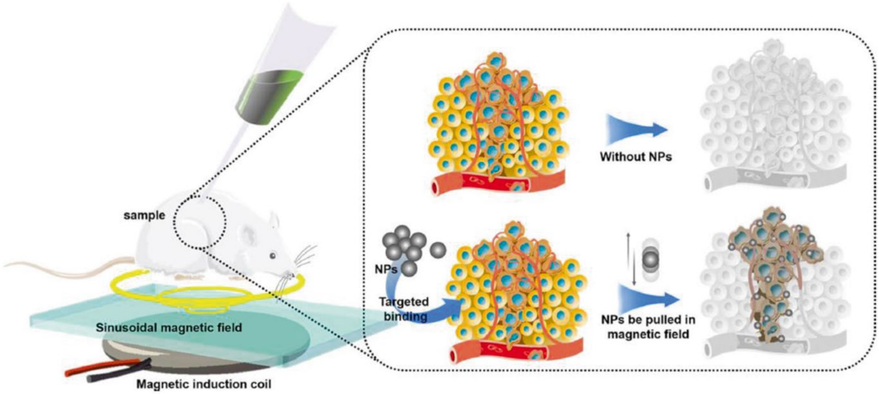


Fig. 2 Diagrammatic illustration of enhancing the contrast level of imaging by nanoparticles [131]

[16]. Various nanomaterials that are used along with conventional imaging techniques are discussed in this section.

Gold Nanoparticles (AuNPs)

Gold nanoparticles are extensively used in theragnostic applications owing to their high atomic number, small size, high specific surface area, surface plasmon, resonance stability properties, surface chemistry, and biocompatibility [3, 36, 69]. They are highly detectable, even at concentrations as low as 10^{-6} M [3]. Their use is the most effective way of imaging tumours as they are easily detected even by a simple optical microscope equipped with a dark field condenser. These AuNPs can resonantly scatter visible and near-infrared radiation due to their surface plasmon resonance (SPR) when their surface plasmon oscillation is stimulated. This release of photons in all directions by the scattering phenomenon serves as contrast agents for intracellular trafficking visualization in dark field optical microscopy. AuNPs, which can be synthesized in various shapes and sizes (Fig. 3), are the most effective for imaging when their sizes range from 30–100 nm [14].

AuNPs are used in photoacoustic tomography (PAT), light, and ultrasound-based hybrid imaging techniques which create a higher level of photoacoustic signals in the NIR regions with enhanced contrast images. Kim et al. [62] used gold nanocages modified with targeting molecules, specifically [Nle^4, D-Phe^7] melanocyte-stimulating hormone, exhibiting a 3.5-fold increase in accumulation compared to untargeted PEG-nanocages. Moreover, they accumulated 3.6 times more than the non-targeted PEGylated gold nanocages control in B6 melanoma tumours and delivered a 300% enhancement in contrast 6 hours post-injection *via* the tail vein in mice (Fig. 4). Moreover, gold nanoparticles can exhibit excellent biocompatibility without *in vivo* accumulations [66] and can find wide applications in cancer theragnosis.

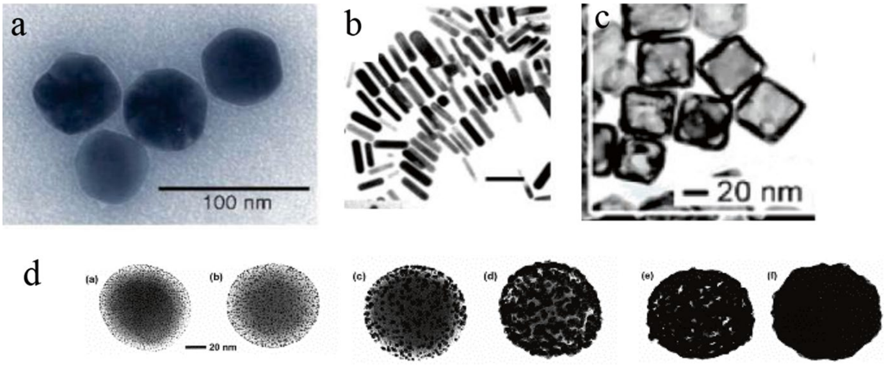


Fig. 3 Various types of gold nanoparticles; nanospheres (**a**), nanorods (**b**), nanocages (**c**), and nanoshells (**d**) (stages of growth of nanoshells on silica nanoparticles) [26, 86, 89, 98]

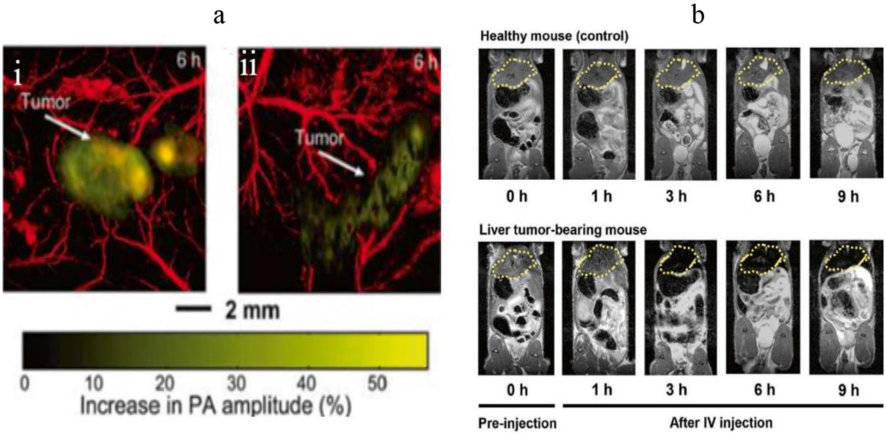


Fig. 4 PAT of targeted gold nanocages demonstrated higher photoacoustic amplitude (i) than non-targeted gold nanocages at the tumour site 6 hours after intravenous injection (ii) (**a**) [62]. MR images of the healthy mouse (top) and a mouse bearing a liver tumour using superparamagnetic gold nanoparticles as a contrast agent (bottom) (**b**) [66]

Quantum Dots (QDs)

Quantum Dots are semiconducting, light-emitting nanocrystals with unique optical properties [104]. QDs are highly resistant to photobleaching [21], broadband absorption, narrow emission bands in UV to NIR region [11, 61], and size-tunable emission bands [96]. A QD with conjugated biomolecules can be used not just for imaging but also for targeting and drug delivery [43]. To make such a system an aptamer (oligonucleotide or peptide molecules that bind to a specific target molecule) that recognizes prostate-specific membrane antigen (PSMA) is conjugated with QD [10] and Doxorubicin (DOX), an anticancer drug with fluorescent property is intercalated in the conjugate. Initially, the fluorescence of both DOX and QD is quenched by the intercalated DOX within the aptamer. When the aptamer binds to its target, DOX is released inside the cell through endocytosis. At the same time, the fluorescent property of QD and DOX is also recovered and can be imaged [30]. Figure 5 illustrates the general working mechanism of QDs. Thus, QDs integrated with imaging, targeting, and therapeutic agents can provide better results in combatting cancer [43].

Among three common QDs based on carbon: graphene quantum dots (GQDs), nanodiamond and carbon dots (CQDs), CQDs of 2 to 20 nm sized fluorescence nanomaterials have drawn more attention because of their multiple attributes such as ease of synthesis, low-cost, strong multi-colour luminescence properties, excellent aqueous dispersibility, tunable optical properties, and biocompatibility [82]. CQDs with different compositions, structures, and properties have been prepared by several cost-effective, portable, and size-controlled methods and are used for drug loading, chemical probes, and phototherapy. Various functional groups on CQDs

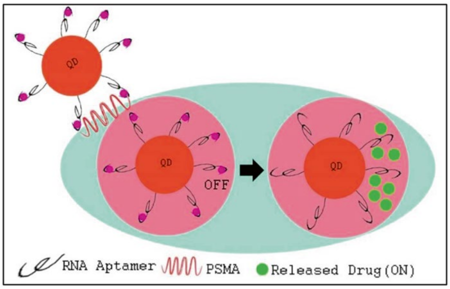


Fig. 5 The working mechanism of QD showing binding of QD with Prostate Specific Membrane Antigen (PSMA) and its subsequent uptake to Lysozyme. After getting released from RNA aptamer, the drug Doxorubicin (shown as pink circles) starts to emit fluorescence (shown as a green dot) (Figure not to scale)

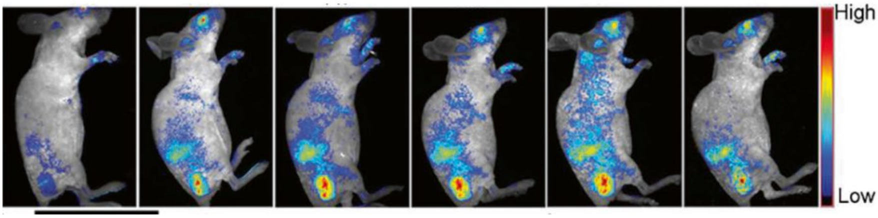


Fig. 6 Real-time in vivo red FL images after i.v. injection of CQDs in nude mice at different time points [45]

have the potential to combine with different metal ions making them suitable for chemical probes. They exhibit strong two-photon luminescence when stimulated in NIR spectrum making them exceptionally suitable for biomedical imaging in deep tissues. This is attributed to their minimized phototoxicity and improved penetration capabilities without background light interference [130].

They can find *in vitro* and *in vivo* applications as a contrasting agent in photoacoustic, fluorescence, and magnetic resonance imaging. Ge et al. [45] investigated the feasibility of CQDs for *in vivo* fluorescence imaging by taking HeLa tumour-bearing nude mice. These mice were injected intravenous (i.v.) injection of CQDs and Fig. 6 shows the accumulation of CQDs in the tumour region providing significant fluorescence compared to other tissues through the phenomenon of enhanced permeability and retention (EPR).

Carbon Nanotubes (CNTs)

Carbon nanotube is another nanomaterial used for the detection of biomarkers. It is a carbon cylinder containing benzene rings that act as sensors for detecting DNA and protein. CNTs are used to differentiate different proteins from serum samples and as carriers for drug delivery [49]. The unique mechanical, electrical, and thermal characteristics of CNTs make them potentially effective tools for detecting the occurrence of proteins indicative of the early stage of cancer [32]. They are extensively used in three imaging modalities: photoacoustic imaging (PAI) [59], fluorescence imaging (FI) [125], and Raman imaging (RI) [124]. CNTs can be used as multifunctional bio-probes with several qualities, including strong NIR absorbance and good resonance, which have drawn increased interest in their usage in cancer imaging for the diagnosis of various cancer types [122].

The detection by CNTs is based on molecular recognition events and is label-free. The greatest advantage of this is the elimination of possible hazards resulting from the use of labeled materials [30]. For the Prostate Specific Antigen (PSA) detection, single-wall carbon nanotubes with $[Ru\text{-}(bpy)_3]^{2+}$-doped silica nanoparticles have been studied. Enough significant electro-chemiluminescent signal is obtained by the nanoparticle system which can be detected with a simple measurement system [70]. Likewise, the glass carbon electrode modified with MWCNT (CNT-GCE) immunosensor integrated with a microfluidic system has been proposed as a promising technique for identifying the expression of typical biological components during the early stages. The high sensitivity and its ultralow detection properties made it superior to the conventional spectrophotometric detection Enzyme-Linked Immunosorbent Assay (ELISA) approach, indicating that it has greater potential to detect prostate-specific antigen (PSA) in the clinical diagnosis of prostate cancer [92].

CNTs have been used as a contrast agent in PA imaging technology, particularly with an infrared laser, due to its broad optical absorption in the visible-infrared spectrum. It has been discovered that CNTs produce a higher PA signal than red ink, even at low laser irradiation intensities [114]. The strong Raman signals of Single-Walled Carbon Nanotubes (SWNTs) are used to identify cancer cells and to selectively destroy breast cancer cells using photothermal ablation as well [128]. The multifunctional nanoprobe consisting of doxorubicin-functionalized SWNTs, hyaluronic acid-modified, and gadolinium-loaded served as the potential for cancer theragnostic which demonstrated excellent biocompatibility and better contrast for MRI imaging [54]. The researchers discovered that when carbon nanotubes are coupled with a metal, such as gold, their efficiency in cancer theragnostic improves significantly. Gold nanoshells enhance the surface-enhanced Raman scattering (SERS) effect by attaching silver or gold to CNTs, enhancing the imaging of targeted cells due to their NIR absorption capabilities [124].

Antaris et al. [8] injected CNT chirality (6,5) after proper mixing with a biocompatible surfactant at a very low dose (4 μg) into the mouse. Figure 7 shows the accumulation of CNTs and the NIR emission over the time of injection that locates the tumour in the mouse.

Thus, CNTs are extensively studied for their potential use in biological imaging technologies, with promising findings in early cancer detection using advanced imaging techniques based on CNTs.

In addition to the above-discussed nanomaterials, other metals and metal oxides also have made tremendous advances as imaging contrast agents in recent decades, which play an important role in the precise and successful diagnosis of medical

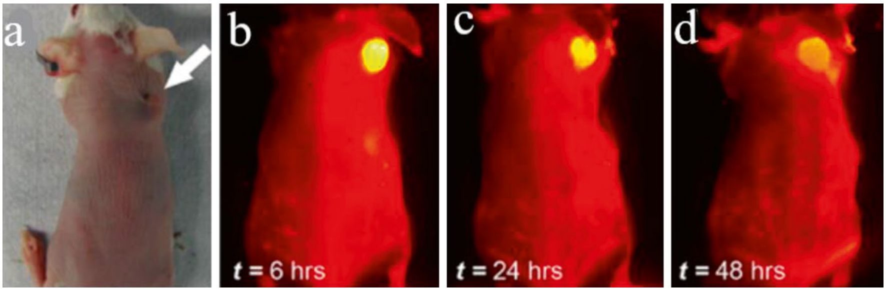


Fig. 7 Optical image of a mouse with a tumour at the right shoulder (**a**), NIR-II fluorescent time course imaging at 6, 24, and 48 hours post-injection showing accumulation of SWCNT on the tumour (**b**–**d**) [8]

conditions. Gao et al. [44] synthesized silver nanoclusters and employed them for imaging xenografted tumours in nude mice. The nanoclusters exhibited strong NIR fluorescence allowing for clear and selective imaging of healthy and tumour cells. The various types of metal oxide nanomaterials such as iron oxides, gadolinium oxides, manganese oxides, tungsten oxides and bronzes, molybdenum oxides, and titanium oxides with tunable morphology are used as imaging contrast agents in MRI and photothermal therapy (PTT), CT and radiotherapy [7].

3 Treatment of Cancer

A multimodal approach is used in the treatment of cancer after its diagnosis, considering the health conditions of the patient, nature, and stage of cancer. Various conventional and nanotechnology-based modalities and their combination are practiced during cancer management, with each modality enriched with its peculiar features and principles. The basic principle of cancer treatment, however, involves destroying cancerous cells while minimizing damage to healthy cells or tissues [33]. The conventional modalities have now been more or less replaced with nanotechnology-based modalities. Nanotechnology offers innovative approaches to cancer therapy and has thus become a propitious frontier in the realm of cancer management.

3.1 Conventional Modalities

Conventional modalities of cancer therapy include homeopathy, Ayurveda, surgery, chemotherapy, and radiotherapy. Homeopathy, having very few adverse effects, is one of the most widely adopted complementary and alternative medicines in the world for treating and managing different types of cancer. Although the mechanism of operation is uncertain, it is believed that homeopathic treatments stimulate the natural defense system and self-healing mechanisms of body. Various cancer cell lines and animal models have been used to validate the action of a few homeopathic drugs. As homeopathic formulations are often diluted beyond Avogadro's number, no pharmacological action can be expected and may be nothing more than a placebo response. Moreover, it was found to be more significant as an adjunct therapy for the treatment of cancer [76, 80].

The symptoms of "*Arbuda*" (inflammatory tumour) and "*Granthi*" (benign glandular enlargement), as mentioned in the classical scriptures of Ayurveda, are similar to those of cancer [9]. In Ayurveda, any form of illness, including cancer, is supposed to occur due to an imbalance between the mind, body, and spirit. Exploring the root cause of an illness is the prime objective of Ayurvedic therapy. So, Ayurveda aims to prevent and treat diseases by restoring balance between the mind, body, and spirit through natural methods such as diet, lifestyle, herbal formulations, yoga, and meditation [12]. Various approaches like *panchakarma* (detoxification of the body), *rasayana* (rejuvenation of cells), *shaman chikitsa* (curative treatment using medicines like *bhasma* and herbal decoctions), and *satwavjay chikitsa* (yoga and meditation) have been suggested in Ayurveda for the prevention and cure of cancer. Surgical management of swellings or tumours has also been suggested in Ayurveda when all other approaches fail. However, no scientific validation can be found regarding the success of those approaches, so they can be used in conjunction with other therapies as adjuvants [101].

Surgery is one of the primitive methods of curing cancer, especially for early-diagnosed local and non-metastatic tumours. It requires assessment techniques such as imaging and biopsy of cancerous tissue repeatedly [100]. There is, however, a high chance of recurrence, metastatic problems, and post-surgery complications [123].

Chemotherapy is one of the most currently available common methods to cure cancer, especially benign and early malignant tumours [127]. It makes use of cytotoxic drugs (injected or taken orally) such as docetaxel, cabacitaxel, doxorubicin, and carboplatin to kill cancer cells. They are considered systemic treatment because the drug molecules are disseminated throughout the bloodstream and affect the entire body system [127]. The chemotherapeutic drugs leave inevitable side effects such as vomiting, nausea, diarrhoea, constipation, hair loss, impaired liver, impeded kidneys, and restrained bone marrow problems to the patient. [106].

Radiotherapy is one of the most effective methods of curing cancer that use radiation such as alpha-, beta-, and proton beams to treat tumours by damaging their DNA. It is relatively safe and painless for the patient. Clinical data has revealed that radiotherapy offers better control and longer survival of many patients compared to other methods [25]. Nevertheless, radiotherapy has its own set of limitations and risks such as weakness, fatigue, local normal tissue damage, and skin itching [72].

3.2 *Nanotechnology-Based Modalities*

Nanotechnology can be used to overcome the limitations of conventional modalities of cancer treatment. Without causing any harm to the healthy cells, nanotechnology provides a promising approach to treating malignant cells in considerably lower doses of drugs [35]. The high surface-to-volume ratio, surface plasmon resonance, minute size, unique morphology, and biocompatibility of nanomaterials make them suitable materials for combating cancer. The high surface-to-volume ratio of nanomaterials facilitates their surface functionalization with various biomolecules so that it could improve target-specific drug delivery and site-specific drug release in a controlled manner [135].

Target-specific drug delivery, either passively or actively, is one of the key advancements of nanomaterial-based cancer treatment over free drugs. Passive targeting utilizes enhanced permeability and retention (EPR) effect while active targeting involves conjugation of bioactive molecules such as antibodies and peptides. The target-specific drug delivery displays enhanced specificity, reduced cytotoxicity, and better loading capacity. Owing to the prominent advances in cancer pathology, many nanomaterials have been developed and applied for cancer treatment but very few of them have been intensively studied and utilized in clinical practice [22, 29, 135]. Some of such drug delivery nanoparticle systems include polymeric nanoparticles, lipid-based nanoparticles, nanoemulsions, dendrimers, carbon nanomaterials, and metallic and magnetic nanoparticles (Fig. 8). These drug delivery systems are briefly discussed in the following paragraphs.

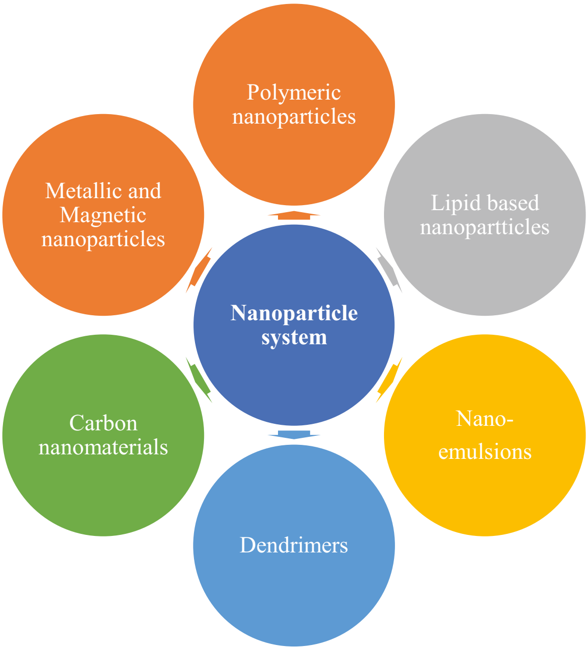


Fig. 8 Drug delivery nanoparticle system in cancer treatment

Polymeric Nanoparticles

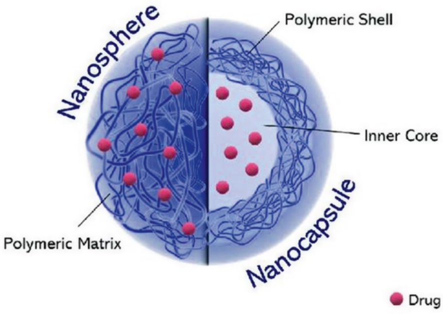
Polymeric nanoparticles (PNPs), 10–1000 nm in size, are colloidal molecules able to carry, transport, and release chemical drugs to targeted malignant cells in a sustained way [77]. The drugs are either dissolved, encapsulated, or covalently bonded to the polymer matrix of PNPs forming a nanocapsule or a nanosphere (Fig. 9) [41, 102]. Natural polymers such as albumin, chitosan, alginate, heparin, and gelatine, and synthetic polymers such as polylactic acid (PLA), poly (lactic-co-glycolic acid) (PLGA), poly (amino acids), poly (ε-caprolactone) (PCL), and polyethylene glycol (PEG) are being widely used to make delivery system [38]. The drugs in the polymeric nanoparticle can either be incorporated during their preparation or through adsorption by incubating polymer in them [119]. They can target specific cancer cells through ligand-receptor, antibody-antigen interaction, or aptamers [93] and the release of drugs is mainly through diffusion. The newer methods to release drugs into the cell use stimuli like radio frequency magnetic field [34] and pH [71]. The magnetic field causes localized heating of the particle and the drugs are released by polymer degradation. This method of sustained drug release has been found to be more effective causing the death of 60% of cancer cells [34].

Lipid-Based Nanoparticles

Research on three different categories of lipid-based nanoparticles: liposomes, solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs) has been extensively carried out [5]. Liposomes are small and spherical vesicles, 20 nm to more than 1 μm in size, and composed of amphipathic molecules like phospholipids (Fig. 10) [52].

A typical liposome consists of a hydrophilic aqueous core and an outer hydrophobic phospholipid bilayer. The hydrophilic aqueous core can encapsulate hydrophilic drugs and the hydrophobic bilayer can bind hydrophobic drugs (Fig. 11). Drugs enclosed within the central core of the liposome are thus protected from the fluid environment during circulation through the bloodstream inside the human body [4, 97, 133]. The membrane of the liposomes might contain different molecules like receptors, antibodies, transport proteins, and lipids. Their ability to incorporate ligands like aptamers, antibodies, peptides, and cytokinin in their surface makes them a unique carrier system. The ligands incorporated on the surface help to actively target only the cancerous cells [58] leaving the healthy cells unaffected.

Fig. 9 Schematic representation of polymeric nanoparticles [41]



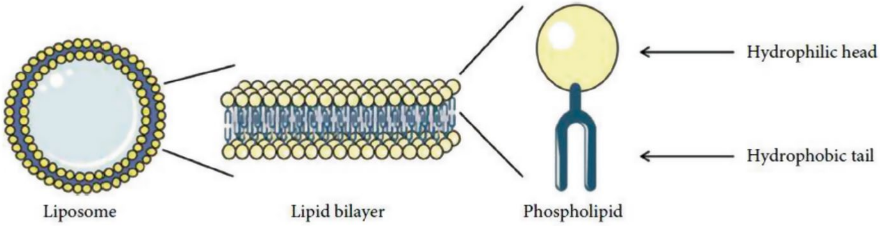

Hydrophilic head
Hydrophobic tail
Liposome
Lipid bilayer
Phospholipid

Fig. 10 Diagrammatic sketch of liposome [4]

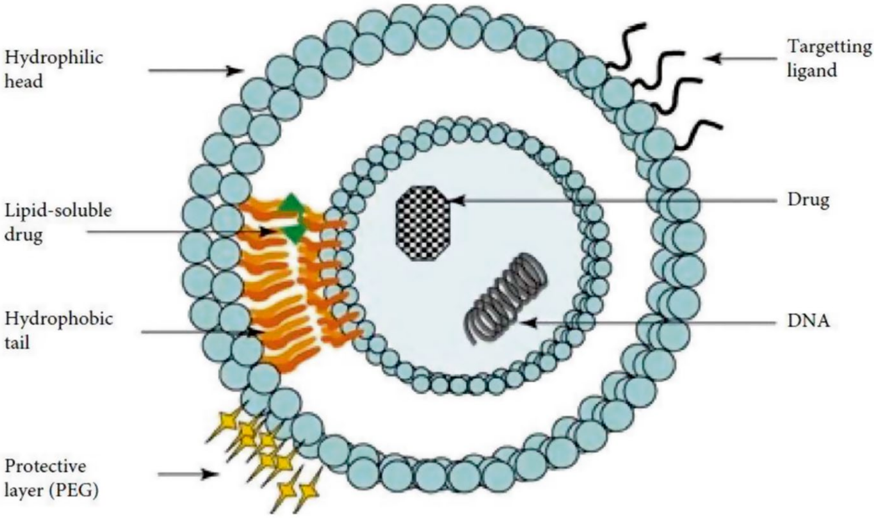

Hydrophilic head
Targetting ligand
Lipid-soluble drug
Drug
Hydrophobic tail
DNA
Protective layer (PEG)

Fig. 11 Diagram illustrating a liposome entrapping both hydrophilic and hydrophobic drugs [4]

Mechanism of Action of Liposomal Drug Delivery

Liposomal drugs, the surface functionalized and anti-cancer drug-loaded liposomes, are administered inside the human body through oral route, intravenous injection (I.V.), or local injection. These drugs travel to different tissues inside the body through the bloodstream. Usually, the voids of normal cells are less than 30 nm while those of tumour cells lie between 80 nm to 200 nm. So, liposomal drugs with suitable particle sizes spontaneously reach and start accumulating at the tumour site without entering healthy cells. This is called the enhanced permeability and retention (EPR) effect. The accumulated liposomal drugs enter tumour cells by endocytosis, the ingestion of materials by the cells through the plasma membrane. Under the influence of the internal environment of tumour cells liposomes release drugs and act on tumour cells to achieve the purpose of cancer treatment (Fig. 12) [53].

A variety of anti-cancer drug-encapsulated liposome formulations have been clinically investigated in recent years for the treatment of different cancers. The next generation of lipid-based nanoparticles, such as solid lipid nanoparticles (SLNs),

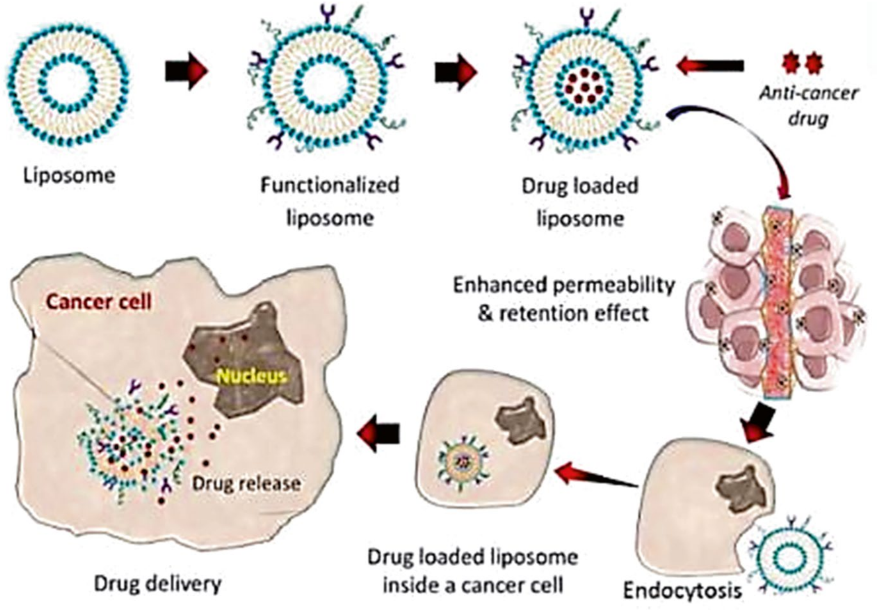

Liposome
Functionalized liposome
Drug loaded liposome
Anti-cancer drug
Enhanced permeability & retention effect
Cancer cell
Nucleus
Drug release
Drug delivery
Drug loaded liposome inside a cancer cell
Endocytosis

Fig. 12 Schematic of liposomal drug delivery system for cancer therapy [53]

nanostructured lipid carriers (NLCs), and lipid polymer hybrid nanoparticles, have also emerged along with the advancements in liposome technology. These particles are expected to address the existing drawbacks associated with liposomes. Unlike liposomes, SLNs are zero-dimensional nanocarriers and NLCs are improved generation of both liposomes and SLN [83].

Advantages of Lipid-Based Drug Delivery System

Studies have reflected that lipid-based drug delivery systems exhibit many advantages when applied to cancer therapy such as minimizing toxic side effects of drugs, enhancing the bioavailability of hydrophobic anti-cancer drugs, providing targeted drug delivery, improving multi-drug resistance, protecting drugs against their surrounding environment, and prolonging the systemic circulation time of delivered anti-tumour drugs [91].

Nanoemulsions

Nanoemulsions are thermally and kinetically stable dispersions, 10 to 1000 nm in size, made by mixing two immiscible phases: usually water and oil, and stabilized by surfactants. The lipophilic core of the dispersion acts as a site for hydrophobic materials and the outer hydrophilic phase can bind hydrophilic materials (Fig. 13) [57, 94]. There may be three types of nanoemulsions systems: (a) water in oil, (b) oil in water, and (c) bi-continuous. Compared with lipid-based nanomaterials, nanoemulsions have greater optical transparency, good thermodynamic and kinetic stability, better drug loading ability, enhanced biodegradability, and sustained drug release tendency [48].

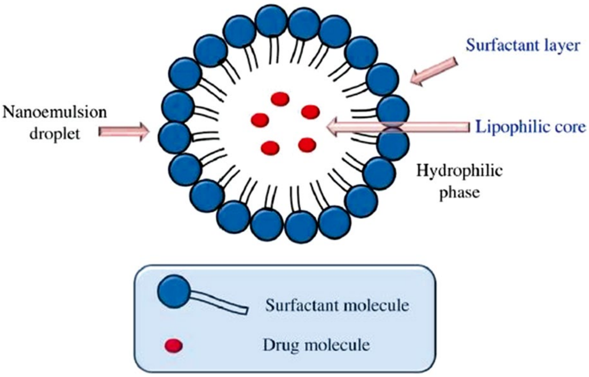
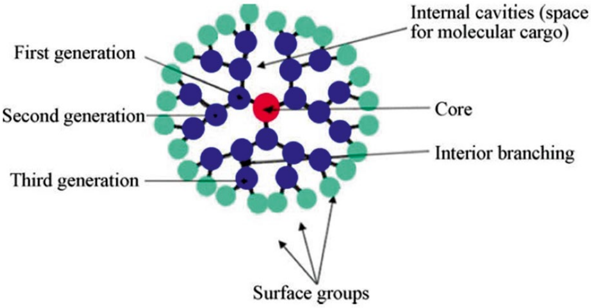

Surfactant layer
Nanoemulsion droplet
Lipophilic core
Hydrophilic phase
Surfactant molecule
Drug molecule

Fig. 13 Schematic representation of nanoemulsion [94]

Fig. 14 Structure of dendrimers [56]


Internal cavities (space for molecular cargo)
First generation
Second generation
Core
Interior branching
Third generation
Surface groups

Nanoemulsions can carry, transport, and release chemotherapeutic drugs to malignant sites in a controlled way without causing any harm to normal cells and organs. The lipophilic anticancer drugs have displayed several benefits when added to oil-in-water nanoemulsions. The drugs get dissolved in an emulsion system when the oil phase of the emulsion acts as a solubilizer for the lipophilic molecule [64].

Dendrimers

Dendrimers are radially symmetrical and highly branched artificial three-dimensional molecules of size ranging from 1 to 10 nm with defined molecular weight and entrapment properties [28, 112]. They have three distinctive architectural components: an interior central core, a middle layer composed of several generations, and an exterior attached to the outermost generation of the middle layer (Fig. 14). The three-dimensional architecture and unique properties of dendrimers have made them excellent carriers for drugs and bioactive agents [56]. The drugs are carried in dendrimers either by entrapping them in the core through hydrogen bonds, hydrophobic and electrostatic interactions [31] or by chemically bonding them through covalent conjugation to the terminals of dendrimers [20].

Several dendrimers such as polyamidoamine (PAMAM), PPI (polypropylene imine), PEG (poly(ethylene glycol), TEA (triethanolamine), and 5-ALA

(5-aminolevulinic acid) have been developed for cancer treatment. The tunable properties of dendrimers have allowed to design and modify them in several ways to produce several different molecules with desired properties and functions [75].

Mechanism of Action of Drug Delivery Through Dendrimers

As mentioned above, dendrimers can act as potential drug carriers either by entrapping anti-cancer drugs to their interior core or by attaching drugs to their surface through different physical or chemical forces. The administration of drugs-loaded dendrimers inside the human body, and their entry inside tumour cells is similar to liposomal drugs. Once the drug-loaded dendrimers enter the tumour cells drug is delivered by either of two mechanisms (Fig. 15).

(a) *In vivo* degradation of drugs-dendrimers covalent bond by suitable enzymes.
(b) Stimuli (pH, temperature) induced release of the drug from dendrimer [95].

Carbon Nanomaterials

Carbon nanomaterials (CNMs) are carbon-based nanosized materials with unique but different properties, morphology, functions, and applications in many industries and medical sectors. These are considered more biocompatible and safer than metal-based nanomaterials in the management of cancer [107]. The inherent hydrophobic quality of CNMs allows the effective loading of drugs through π-π stacking or hydrophobic interactions for sustained delivery [90]. The role of several CNMs (Fig. 16) such as graphenes, carbon nanotubes (CNTs), and carbon quantum dots (CQDs) have been hugely investigated in cancer treatment.

Graphene is a two-dimensional crystalline form of carbon with sp^2 hybridized carbon sheet (Fig. 16c) and extraordinary features. Graphene and graphene-based nanomaterials such as single-layer graphene, multi-layer graphene, graphene oxide,

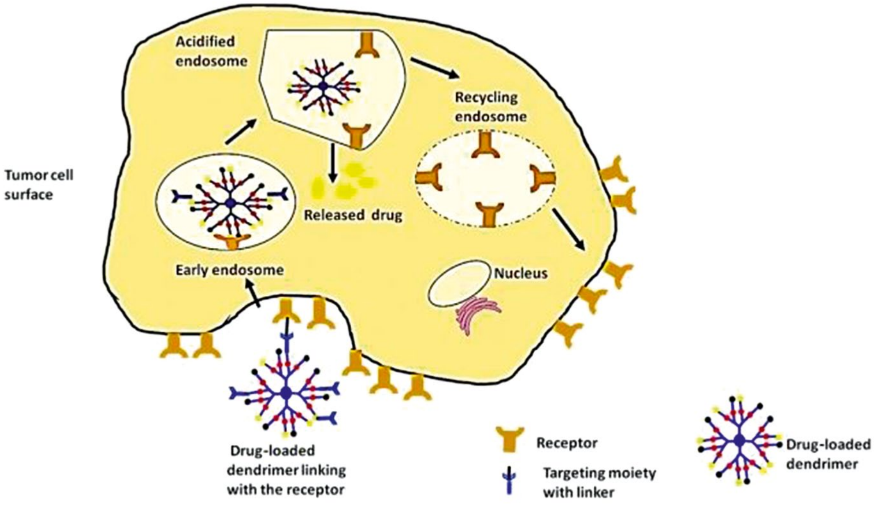


Fig. 15 Schematic showing the mechanism of targeted delivery through dendrimers [95]

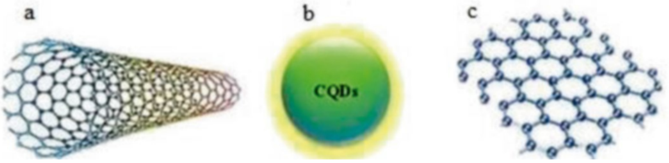


Fig. 16 Carbon nanomaterials CNTs (**a**), CQDs (**b**), and Graphene (**c**) [78]

and reduced graphene oxide have unique electrochemical and mechanical properties like optical clarity, inertness, high density, greater hydrophobicity, high drug-carrying abilities, and thermal conductivity which allow them as a potential carrier in cancer theragnostic [74]. Graphene oxide contains many oxygen-bearing functional groups, such as hydroxyl, carboxylic acid, and epoxide groups on the carbon surface which makes it more hydrophilic. Graphene layers have been incorporated into nanocomposites to control and improve surface area, mechanical properties, electrical properties, and thermal conductivity [108].

CNTs are graphene sheets wound up into a seamless cylindrical tube (Fig. 16a). Owing to their high aspect ratios, exposed surface areas, rich surface functionalities, and minute size they have received attention as carriers to transport anticancer drugs and as mediators for photothermal and photodynamic cancer therapy to directly destroy cancer cells [118]. Among two varieties of CNTs [73], single-walled CNTs have more clearly defined walls and are found to be more effective at delivering drugs than multi-walled CNTs SWNTs [37]. These can also interact with immune cells and induce an immune response to suppress the growth of malignant tumours [79]. Chen et al. [27] developed biotin-functionalized single-walled CNTs conjugated with taxoid, an anticancer drug, using a cleavable linker. The drug was delivered to the targeted site via endocytosis, released inside the cell, and interacted with microtubules which was assessed by flow cytometry. This interaction led to the formation of a stable microtubule-taxoid complex ultimately causing apoptosis and the death of cells (Fig. 17).

Similarly, CQDs (Fig. 16b), featuring exceptional water solubility and biodegradability, have been effectively employed in drug delivery systems as anti-cancer nano-drug carriers, in photodynamic (PDT), and photothermal (PTT) therapy of cancer, thereby shifting the paradigm of cancer management [67]. By using microwave synthesis to create CQDs from citric acid and urea, Zeng et al. were able to create a drug combination of CQDs and doxorubicin (DOX) with superior surface chemistry in which the amine group of the drug and the carboxyl group of CQDs bonded via hydrogen bonding. This non-covalent interaction differentiates cancerous and normal cells based on their pH difference and kills cancer cells effectively. CQDs protect normal cells from toxicity while increasing the intracellular concentration of drugs in cancer cells [132]. In the photodynamic approach for cancer treatment, the interaction between light of definite wavelength and photosensitizing agents is used to produce reactive oxygen species which kills cancerous cells by oxidative DNA damage. However, the photosensitizers are reported to have limited solubility and poor selectivity which can be increased by using CQDs based carrier

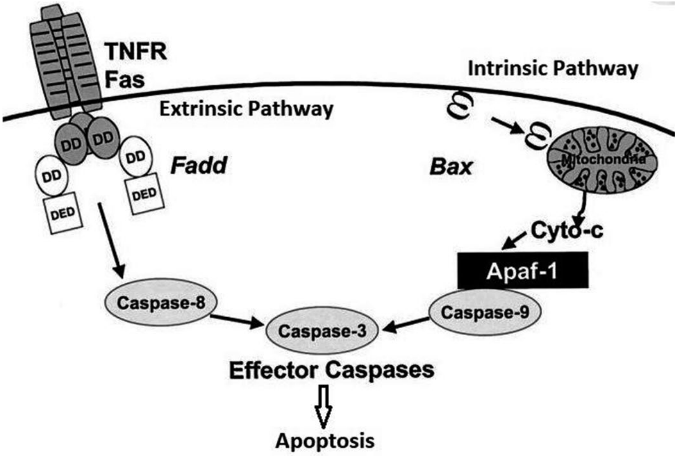


Fig. 17 Schematic of caspase activation pathways and apoptosis. (Adapted from Ref. [103])

system. Similarly, in photothermal therapy, near-infrared light has been used to generate heat and kill cancerous cells by raising tissue temperature. Kim et al. synthesized sulfur-doped CQD with 55.4% photothermal conversion efficiency from *Camellia japonica* flowers [63].

Metallic and Magnetic Nanoparticles
Metallic nanoparticles have been widely investigated in cancer therapy using different approaches such as drug delivery, PDT, and antioxidants owing to their distinct optical, magnetic, high surface-to-volume ratio, and photothermal features. One of the unique properties displayed by metallic nanoparticles, typically gold, silver, titanium, zinc, and copper NPs, is surface plasmon resonance (SPR). This property has been used to design near-infrared responsive metal nanoparticles that can acquire photothermal agents for the treatment of tumours [17]. The exceptional optical and physical properties of a variety of gold nanoparticles, including gold nanorods, nanostars, nanocubes, nanocages, and nanospheres, have allowed them useful in the management of cancer [2]. Furthermore, gold nanoparticles are non-toxic, highly permeable, and have good retention effects which make them more likely to invade the cancerous cells and produce better therapeutic effects [111]. Nirmala et al. successfully synthesized gold nanoparticles stabilized with Vitis vinifera skin polyphenols that can induce apoptosis by increasing reactive oxygen species production in the A431 skin cancer cell line [87].

Magnetic nanomaterials (MNPs) are an imperative class of nanoparticles that are fabricated from pure metals like iron, cobalt, nickel, and some rare earth metals or a mixture of metals and polymers. MNPs such as iron oxide nanoparticles possess good magnetic moments and high surface-area-to-volume ratios that make them attractive for hyperthermia-based cancer therapy and target-specific drug delivery [39]. When guided by the external magnetic field, MNPs loaded with chemical drugs can target cancer cells by mitigating the side effects of those drugs [134].

Mechanism of Action of Cancer Therapy Using Metallic Nanoparticles
Metallic nanoparticles display a multifaceted approach to cancer therapy by inducing cancer cell apoptosis, drug delivery systems, or localized heating without causing any harm to the healthy cells. The metallic nanoparticles functionalized with suitable biological molecules (ligands, peptides, antibodies, nucleic acids, and so on) are specifically delivered to the tumour site where they bind to cancer cell receptors and enter the cell through endocytosis. Inside the cell, these metallic nanoparticles induce oxidative stress by releasing reactive oxygen species (ROS). The release of a huge amount of ROS inside the cell causes mitochondrial dysfunction, and damage to cellular components including DNA thereby activating apoptotic pathways [23].

Apoptosis, a programmed cell death, is an orderly molecular event essential for the development and homeostasis of tissues. It involves two main pathways: the intrinsic (or mitochondrial-mediated) pathway and extrinsic (or death receptors-mediated) pathways. The intrinsic pathway is triggered by internal stimuli such as DNA damage, oxidative stress, or lack of growth factors that result in the mitochondrial outer membrane permeabilization (MOMP), the opening of mitochondrial transition core, and subsequent release of cytochrome c into the cytoplasm. Cytochrome c binds with Apaf-1 and forms an apoptosome that activates a cascade of caspases resulting in apoptosis which is characterized by features such as chromatin condensation and DNA fragmentation. On the other hand, the extrinsic pathway is triggered by the interaction of extracellular ligands (for instance, members of the TNF family of cytokine receptors, such as TNFR1 and Fas) with their respective death receptors on the surface of the cell. This interaction activates a cascade of caspases resulting in apoptosis (Fig. 17) [103].

Metallic nanoparticles have also been used as nanocarriers in cancer therapy by loading them with anti-cancer therapeutic agents (or drugs) and designed for controlled and sustained release of loaded drugs in response to the pH, temperature, or enzymes within the tumour microenvironment [1].

Researchers have also used metallic nanoparticles for hyperthermia (localized heating) based cancer therapy. Gold nanoparticles have been used in photothermal therapy in which these particles are irradiated with near-infrared (NIR) light to generate localized heat. The heat energy selectively destructs cancer cells by raising the temperature within the tumour microenvironment. Similarly, iron oxide nanoparticles have also been used to produce localized heat enough to kill cancer cells by exposing them to an alternating magnetic field [129].

4 Opportunities and Challenges

Nanomaterials offer promising opportunities in the theragnostics of cancer. The success of cancer treatment greatly relies on the early detection of cancer. Nanoparticles can be engineered to target the specific biomarkers associated with cancer cells that allow detection *via* various imaging techniques. In addition to this, nanoparticles can be designed as therapeutic agents or drugs for cancer cells without affecting healthy tissues and reducing the side effects. This reduces toxicity with enhanced and efficient drug delivery systems. Nanotechnology can even tailor cancer therapies to individual patients' genetic profiles and the nature of tumour cells. This allows clinicians to design treatment strategies that are effective causing minimized adverse reactions.

Nanomaterials undoubtedly have enormous potential in the diagnosis and therapy of lethal cancer. However, these nanomaterials face serious issues before using them in real clinical applications. The major problem associated with using nanoparticles is the recognition of NPs as foreign by the immune system. They are treated like viruses by the immune system and rapidly attacked by the cells of the mononuclear phagocyte system (MPS), part of the body's defense against protozoa, bacteria, and viruses. The MPS will shuttle the injected nanoparticles to the liver and spleen which prevents them from reaching the target site and delivering their chemotherapeutic payloads [50].

The next challenge is their design and synthesis of uniform shape and size particles. The properties of nanomedicines can vary per batch even under carefully controlled conditions. Furthermore, the mechanism of systemic toxicity of these nanomaterials within the body has not been fully understood. Various factors such as surface charge, size, shape, ligands, and cell lines impact differently in the microenvironment and their metabolism processes are different. Thus, the *in vivo* mechanism action of nanomaterials is the subsequent challenge [113]. The clinical advancement of nanomedicine encounters various hurdles like biological complexities, ensuring biocompatibility and safety, reproducibility, large-scale manufacturing, navigating governmental regulations, and competing with existing therapies in terms of cost [16].

5 Conclusion and Prospects

Recent advances in nanomedicine have opened promising avenues for combatting lethal cancer through diagnosis with precision, targeted therapy, and personalized treatment approaches. Researchers can develop innovative strategies by taking the leverage of nanotechnology to improve early detection, efficient drug delivery, and monitor the response of treatment for optimum cancer management. As opposed to the conventional treatment methods, the risk of toxicity to normal cells, poor solubility of drugs, and multidrug resistance is also minimized while using nanotechnology. These advancements have the potential to transform nanomedicine into revolutionizing oncology with advanced patient care. However, considerable progress in the theragnostic of cancer through nanomedicine remains to be made. The long-term effect of labeled materials used in nanotechnology and the risk of accumulation of nanoparticles in the system need to be further investigated. Creating multifunctional nanoplatforms offers novel routes and strategies for advancing clinical cancer nanomedicine in the future. These platforms also have the potential to enhance the effectiveness of both diagnosis and therapies [113]. Clinically acceptable biomarkers that could reliably signal the disease at very low concentrations even to femtogram level can be developed. The immunogenic nature of nanoparticles demands thorough *in vitro* and *in vivo* research including assessments of immunotoxicity and haematotoxicity. Additionally, a better understanding of how the immune system affects targeted drug delivery by the nanoparticles is essential. Moreover, the metabolism of nanomaterials within the body must be understood in detail. Collaborative interdisciplinary research can solve the existing problems of biomedical nanomaterial-based cancer theragnostics. The advancement in cancer treatment using nanotechnology and its worldwide availability at an affordable cost has the potential to convert cancer from a difficult-to-cure disease to an easily cured disease. There is no doubt that in the near future nanotechnology will drastically change the trend in cancer therapy and will significantly decrease the cancer mortality rate.

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Conflicts of Interest The authors declare no conflict of interest.

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Nanomedicine in Targeted Drug Delivery: Precision Therapeutics for Personalized Medicine

Sangita Dey, Sana Hassan, and Rajan Kumar Pandey

1 Introduction to Nanomedicine and Targeted Drug Delivery

1.1 Overview of Nanotechnology in Medicine

Nanotechnology, denoted by the prefix “nano,” involves the study of extremely small structures, typically at the scale of atoms and molecules [1]. The term “nano” originates from the Greek word for “dwarf,” emphasizing the miniature size associated with nanotechnology. The concept of nanotechnology, rooted in Richard Feynman’s 1959 vision, has evolved into a transformative field influencing diverse disciplines [2]. This field focuses on manipulating individual atoms or compounds to design, characterize, manufacture, and create devices and systems with unique properties. This innovation has gained prominence, as highlighted by Winfred Phillips’ emphasis on fabricating, analyzing, and handling extremely small entities. Approaches in nanotechnology include both top-down and bottom-up methods. Top-down involves reducing the size of larger structures, while bottom-up involves manipulating individual atoms and molecules, resembling processes in chemistry and biology.

At the nanoscale, the behavior of individual molecules and their interactions becomes crucial in determining the material or device’s macroscopic properties. Nanoscale refers to dimensions on the order of one-billionth or 10^{-9} meters [3]. The

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bottom-up strategy is demonstrated by methods like molecular self-assembly, sol-gel processing, and AFM. On the other hand, top-down manufacturing uses cutting, carving, and molding to shape materials; yet, it has limits that make it difficult to produce extremely sophisticated nanodevices. Top-down nano-scale material fabrication uses techniques such as laser ablation, nano-lithography, and electrochemical processes. Applications of nanotechnology include everything from nanomedicine to nanoelectronics to nano-concrete, all utilizing elements found in the periodic table. By combining precise nanoscale building pieces into bigger structures with customized features, nanotechnology can revolutionize the fabrication of materials and do away with the need for conventional machining.

Nanotechnology emerged as a scientific field due to the observation that materials developed at the nanoscale exhibit properties distinct from bulk materials. Changes in atomic size, shape, and arrangement directly impact the material's properties, aligning with fundamental principles of physics and chemistry [3, 4]. Nanotechnology in medicine refers to the application of nanoscale materials, devices, and techniques for diagnosing, treating, and preventing diseases. Nanotechnology is considered the future of science and holds tremendous potential to revolutionize medical treatments by providing targeted and efficient approaches at the molecular and cellular levels. The fields of biology, medicine, and nanotechnology have come together during the past forty years to form the field of nanobiotechnology, which is currently making major advancements in several healthcare sectors [5] including personalized medicine, cancer treatment, drug development, illness detection, pharmaceutical advancements, and state-of-the-art medical instruments and methods.

The field of nanobiotechnology has been significant in advancing medical diagnosis and therapy. Nanosensors, nanoparticle-based imaging agents, nanoparticle-based PCR Assays, and Lab-on-a-Chip devices are examples of diagnostic instruments that use nanotechnology to improve the accuracy and efficiency of illness diagnosis. Modern pharmaceuticals and medications use nano-based approaches in their therapeutics, including polymeric nanoparticles, liposomal formulations, and drug delivery vehicles based on nanoparticles. In the field of nanomedicine, two notable examples of these developments are Abraxane (nanoparticle albumin-bound paclitaxel) and Doxil (pegylated liposomal doxorubicin) [6, 7]. Furthermore, nano-biotechnology has expanded into fields such as gene therapy, where nanotechnology is essential for precisely delivering genetic material [6]. The adaptability of nano-biotechnology in treating infectious diseases and advancing public health is demonstrated by the creation of vaccines and antibacterial medicines based on nanoparticles.

As time goes on, medical approval for nanomedicine and nano vaccines is increasing regularly, following the same pattern as traditional immunizations [8, 9]. A revolutionary age in the nexus of nanotechnology and medicine is being heralded by the commercialization of these nano-biotechnological devices, which highlights their promise for both research and therapeutic applications. Using unique physical characteristics at the nanoscale, like the volume/surface ratio, nanodevices function in both extracellular and intracellular environments.

Gold nanoshells for cancer diagnostics and therapy, and liposomes as vaccine adjuvants and drug delivery vehicles are two exciting new applications of nanomedicine that have been successfully tested in mice and are awaiting human trials [1]. In rats, nanomedicine has also shown success in drug detoxification. Nanodevices are a major improvement in medical applications because of their increased speed and sensitivity when compared to traditional medication delivery systems [10] (Fig. 1).

Richard Feynman presented a visionary idea related to manipulating materials at the atomic and molecular levels in his famous 1959 lecture titled "There's Plenty of Room at the Bottom." However, the actual term "nanotechnology" was coined later. The term gained popularity in the 1980s and 1990s as research and development activities in the field [2].

The development of nanotechnology can be outlined as follows:

1959 – Feynman's Vision: Richard Feynman introduces the concept of manipulating materials at the atomic and molecular levels, setting the foundation for nanotechnology.

1974 – Coining of the Term: The term "nanotechnology" became widely recognized and used for the first time by Taniguchi, who began exploring the potential of molecular-level manipulation and assembly.

1981 **–** IBM Scanning Tunnelling Microscope.

1985 – "Bucky Ball".

1986 – Drexler's "Engines of Creation": K. Eric Drexler publishes "Engines of Creation," a book that popularizes the concept of nanotechnology. This work outlines the potential of molecular nanotechnology and its transformative impact on various fields.

1989 **–** IBM logo was made with individual atoms.

1991 **–** Carbon Nanotube was discovered by S. Lijima for the first time.

1999 **–** R. Freitas published a book on "Nanomedicine".

2000 **–** The National Nanotechnology Initiative was launched for the first time.

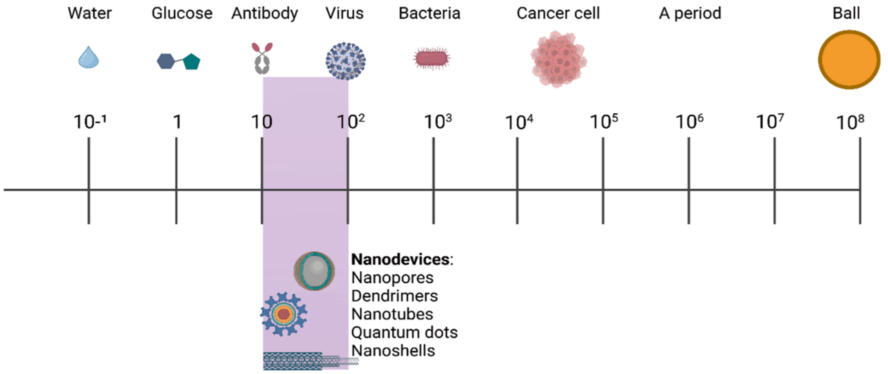

Fig. 1 Comprehensive overview of nanoparticle classes and engineered nanostructures for advanced drug delivery systems

2001 – The Feynman Prize in Nanotechnology was given out in honor of contributions to the synthesis and characterization of carbon nanotubes and nanowires as well as for advances in the theory of nanometer-scale electrical devices.

2002 – The Feynman Prize in Nanotechnology was given for using DNA to help make novel structures and for improving our ability to understand molecular machine systems.

2003 – The integration of single-molecule biological motors with nanoscale silicon devices, as well as the modeling of novel materials' molecular and electrical properties, won the Feynman Prize in Nanotechnology.

2004 – A major milestone was reached with the first-ever policy meeting on advanced nanotechnology. Simultaneously, the first nanomechanical systems center was founded. The Feynman Prize in Nanotechnology was awarded, notably for the development of novel enzymes with modified functions and stable protein structures.

2005–2010 – Robotics, 3D networking, and active nanogoods that may alter their state while in use are all examples of 3D nanosystems that have been produced successfully.

2011 – The molecular nanotechnology era commenced (Fig. 2).

1.2 Evolution of Targeted Drug Delivery Systems

Targeted drug delivery systems have undergone a dynamic and inventive evolution in response to the need to maximize therapeutic efficacy while reducing side effects.

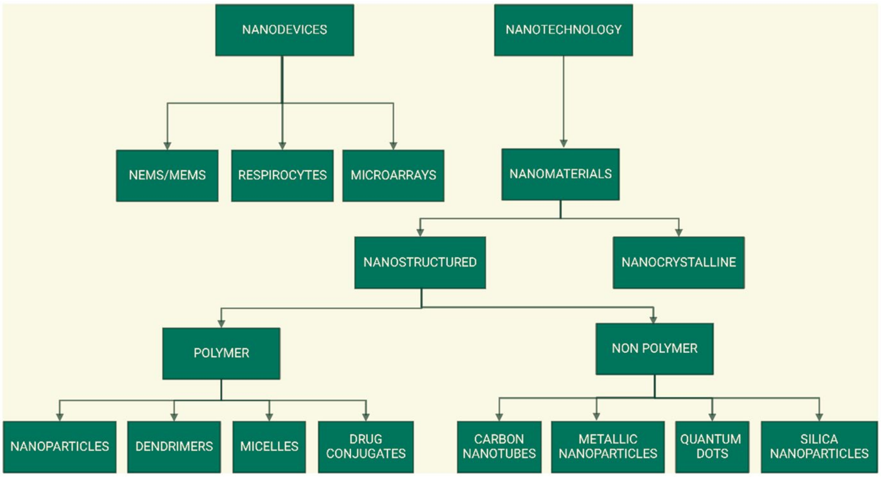
NANODEVICES
NANOTECHNOLOGY
NEMS/MEMS
RESPIROCYTES
MICROARRAYS
NANOMATERIALS
NANOSTRUCTURED
NANOCRYSTALLINE
POLYMER
NON POLYMER
NANOPARTICLES
DENDRIMERS
MICELLES
DRUG CONJUGATES
CARBON NANOTUBES
METALLIC NANOPARTICLES
QUANTUM DOTS
SILICA NANOPARTICLES

Fig. 2 Schematic illustration representing a variety of pharmaceutical nanosystems

Controlled Release Systems (the 1950s–1980s)

In 1952, the 12-hour drug delivery system known as Spansule® sustained-release capsule technology was introduced. The medication delivery industry was dominated by oral and transdermal formulations with therapeutic durations of up to 24 hours.

Implantables and Long-Acting Injectables (1989)

Long-acting injectables and implantables were made possible in 1989. The introduction of Lupron Depot® allowed for the extension of medication delivery from days to months and sometimes even years. Enabled for the long-term delivery of protein and peptide medications, but only by parenteral delivery.

PEGylation Era (1990s)

The PEGylation era began in 1990 with the introduction of Adagen®, a PEGylated protein, which resulted in the creation of siRNA in PEGylated Lipid Nanoparticle (Onpattro®), PEGylated Naloxone (Movantik), and PEGylated Liposomal Doxorubicin (Doxil®).

Nanocrystal Formulations and Drug-Polymer Complexes (2000s)

The release of the iron-dextran complex injectable InFed® in 1974 and the paclitaxel-albumin complex Abraxane® in 2005. Rapamune® (sirolimus nanocrystal formulation) and Mylotarg™ (antibody-drug conjugate-gemtuzumab ozogamicin) were introduced in 2000.

Nanomedicine and the COVID-19 Vaccine Delivery System (2000–Present)

The National Nanotechnology Initiative's launch in 2000, was followed by a significant amount of nanomedicine research. The development of lipid nanoparticle formulations for the COVID-19 vaccination.

1.3 Continuous Evolution and Unfulfilled Needs

Continuous efforts are being made to overcome obstacles such as enhancing water solubility, getting beyond biological barriers, and developing effective long-acting depot formulations. A focus on encouraging a variety of medication delivery research subjects to foster future developments.

Drug Delivery Systems with Controlled Release

A shift from immediate-release formulations to controlled-release systems that maintain drug-release kinetics over time.

Present Patterns: Biomimetic Techniques and Customized Medicine

Personalized medicine and biomimetic techniques for more efficient drug administration are examples of emerging trends.

Recent advancement in drug development is “nanodrugs” where nanoparticles are specifically designed for targeted drug delivery. Drug delivery systems (DDS) have been employed in both clinical and pre-clinical settings to administer therapeutic substances to treat diseases. Drug delivery to particular parts of the body, such as tumors, inflammatory tissues, and contaminated areas, can be done precisely and under control through the use of nanoparticles. Significant progress has been made in drug delivery systems over the last few decades, particularly with the development of micro and nanorobots. These cutting-edge devices are considered superior for efficient drug delivery, utilizing the conversion of various forms of energy into propulsion and controlled movements. Their advantage is that they can more effectively deliver drugs by precisely targeting specific sites within physiological environments and conditions, enhancing the effectiveness of drug delivery [11]. This focused strategy minimizes negative effects linked to wider drug dispersion by lowering the necessary dosage of the treatment [12]. Drug stability and solubility can be improved by using nanoparticles. The overall efficacy of medications in treating a range of disorders is influenced by improvements in solubility and stability. Drugs with improved absorption and distribution throughout the body because of nanotechnology have increased bioavailability. The utilization of lower medication dosages is made possible by this advancement, which eventually lowers the danger of toxicity while preserving therapeutic efficacy [1]. To maximize their efficacy, drug delivery systems are being modified to confirm the principles of nano scaling. Drug-delivery medical nanorobots are being developed. Because these nanorobots can go through veins, medications can be delivered to parts of the body precisely [13]. Antitumoral responses are being studied using nanotechnology, which points to a targeted tumor treatment strategy. To treat cancers and other disorders, researchers are looking at the potential of wireless intracellular and intranuclear nanoscale surgery. The development and testing of respirocytes-mechanical red blood cell technologies represent the main focus of scientific endeavors. Compared to normal red blood cells, respirocytes, a type of nanorobotics, have the ability to provide substantially more oxygen to bodily tissues [11]. The potential application of nanotechnology in the future for the diagnosis and treatment of many diseases associated with blood is increased by the improved oxygen-delivery capability of nanorobotics. The use of nanotechnology in drug delivery as a whole has completely changed the field of medicine. Nanotechnology reduces adverse effects, increases therapeutic efficacy, and allows for efficient and tailored drug delivery. It is expected that further developments in nanotechnology-based medication delivery systems will shape the future of drug delivery [1].

DNA nanotechnology in the realm of drug delivery has introduced innovative approaches, including DNA guns and vaccines. DNA nanotechnology is a rapidly developing area of nanomedicine that enhances drug-targeting precision and mitigates drug toxicity by facilitating the self-assembly of nanostructures and molecules. This is especially promising in addressing toxicity challenges in diseases like cancer, often associated with chemotherapeutic drugs [14]. Recent advancements highlight the adoption of contemporary programming optimization and *in silico* methods for designing DNA nanostructures with precise attributes like size, structure, surface chemistry, and functional properties tailored to specific diseases. The focus extends to creating personalized targeted drug therapies through nanotechnology-based DNA medicine. Notably, efficient drug biomolecules such as doxorubicin and CpG oligonucleotides have been successfully integrated with DNA-based nanostructures to enhance cellular intake efficiency.

The combination of lab-on-a-chip technology with nanotechnology has revolutionized healthcare by providing ground-breaking approaches to medication administration, individualized treatment plans, and disease diagnostics. The outcome of this combination has been the development of advanced diagnostic instruments that outperform conventional techniques in terms of efficiency, precision, and economy. One significant result is the development of Lab-on-Nanoparticles, which are small, multipurpose devices that can be used for medication administration, health condition monitoring, and diagnostics [15]. Made of materials at the nanoscale, these gadgets can sense and react to physiological changes, allowing for real-time tracking and customized care. Advanced medication delivery systems have also been made possible by the application of nanotechnology and Lab-on-a-Chip technology, which has further improved the state of healthcare innovation [16].

1.4 Significance of Precision Therapeutics in Personalized Medicine

The goals of personalized medicine and precision medicine are to tailor medical care to each patient's unique circumstances, including genetics, lifestyle, and surroundings. Using DNA, RNA, and protein analysis, precision medicine analyses genetic and molecular data to pinpoint disease subtypes and create customized treatments. However, to develop a thorough treatment plan that is unique for every patient, personalized medicine takes into account a wider variety of variables, such as genetics, lifestyle, environment, and medical history [17, 18]. Both approaches have gained significance with technological advancements and increased understanding of genetics and disease mechanisms. Nanomedicines are increasingly used in personalized medicine due to their tunable physicochemical properties. The application of nanomedicines has grown significantly, offering the chance to treat particular patient traits by taking their genetic makeup into account. Different kinds of nanomedicines, including liposomes, polymeric nanosystems, magnetic nanoparticles, and nanoparticles, all have advantages over conventional treatments in common [19]. These are benefits that alter the active principles' pharmacokinetics and pharmacodynamics. With its many benefits, personalized nanomedicine appears to be the way of the future for healthcare. Because of its customized approach, side effects can be reduced and therapeutic outcomes can be maximized through precision targeting and therapy. Targeted delivery, regulated drug release, and adaptability to unique patient features are important sub-properties that enhance the efficacy of personalized nanomedicine. Together, these traits improve the effectiveness of treatments, lessen side effects, and open the door for more effective, patient-centered medical interventions [20].

Size Advantage

Nanomaterials can have their surfaces fine-tuned because of their enormous surface-to-volume ratio and compact size. Operating on a nanometric scale provides a foundational advantage, enabling precision in therapeutic applications.

Encapsulation of Activities

Nanosystems make it possible to encapsulate several types of activities, including hydrophilic and lipophilic ones. This versatility enables higher doses that might not be achievable in traditional therapies due to concerns related to solubility. By ensuring that the active compounds reach particular cellular compartments, this encapsulation not only modifies the pharmacokinetics of the compounds but also optimizes their therapeutic effects.

Environmental Protection

Nanomedicines maintain the stability and efficacy of encapsulated activities by shielding them from external elements like light and nucleases.

Controlled Active Release

Pharmacokinetics can be altered to provide a regulated release of active ingredients. The benefit of this regulated release is to prolong the therapeutic efficacy while lowering dosage frequency.

Use of Labile Compounds (e.g., siRNA)

Incorporating delicate compounds like siRNA underscores the flexibility of personalized nanomedicine to accommodate advanced therapeutic strategies.

Targeting Actively

A local therapeutic effect can be facilitated by actively targeting the target organ with nanomedicines. This focused strategy reduces adverse effects while maximizing medicinal efficacy.

Flexibility in Design

The versatility of nanomedicine stems from the ability to choose from various types of nanosystems. This makes it possible to create specialized therapies that are meant to provide individualized therapy. The adjustability and flexibility of these nanocarriers offer a broad spectrum of possibilities for customization, allowing for tailored solutions to specific medical needs [17].

2 Nanocarriers in Targeted Drug Delivery

2.1 Types of Nanocarriers

Liposomes

Liposomes are novel drug delivery vehicles used in pharmaceutical medication delivery systems. They have made a profound impact on chemotherapy by improving selectivity, reducing cytotoxicity, and enhancing the solubility of hydrophobic drugs. Liposome size is important; in vitro and in vivo studies have demonstrated that smaller liposomes (diameter of about 50 nm) generated via microfluidics have superior tissue distribution compared to bigger liposomes (diameter of 75 nm). Liposomes are lipid-based nanoparticles, featuring a closed spherical lipid bilayer structure that forms an inner cavity capable of holding aqueous solutions. Two tightly arranged phospholipid sheets with hydrophobic tails and hydrophilic heads make up the structure. The hydrophobic tails are attracted to each other, forming a double layer that prevents the inner solution from leaking out.

Numerous research on the delivery of chemotherapy have made use of liposomes; one such study used lipofectamine to boost transfection efficiency and decrease chemo-resistant proteins. Magnetic liposomes have also been explored in cancer therapy. A highly stable nano theranostic compound called liposomal IR-780 has been used to treat brain tumors. Moreover, liposomes are used in medication delivery as flexible and biocompatible carriers, especially for the active and passive administration of peptides, proteins, and genes. Research has successfully delivered certain components, such as "Glucose Regulated Protein 78" to cancer stem cells and breast cancer cells by liposome delivery. Furthermore, magnetic liposomes have been used in the field of cancer treatment. Liposomes' small size, biocompatibility, and adaptability make them an attractive drug delivery platform. They are a highly researched and developed nanocarrier in the field of targeted drug delivery because they aid in increasing chemotherapy's efficacy, decreasing toxicity, and boosting drug selectivity.

Polymeric Nanoparticles

Solid materials with diameters ranging from 1 to 1000 nm are represented by polymeric nanoparticles (PNPs), which have two distinct structural forms: nanospheres and nanocapsules. PNPs provide a flexible platform for drug delivery because of their exceptional mechanical strength, electrical conductivity, and optical and thermal characteristics. By microencapsulating these nanoparticles, a low-toxicity, and highly biodegradable drug delivery system can be created. PNPs show promise in protecting therapeutic molecules during delivery and have wide-ranging applications in tissue engineering and vaccine development. Natural polymers are frequently used in drug delivery to help carry a variety of pharmacological substances, such as plasmid DNA, vitamins, antioxidants, antiviral agents, anticancer medicines, and antisense oligonucleotides. PNP synthesis involves the use of synthetic polymers that fall into two categories: biodegradable and non-biodegradable. A smaller percentage of non-biodegradable poly-acrylates than their biodegradable counterparts are used for transdermal and cutaneous medication administration. In transdermal medication delivery, biodegradable polymers such as poly-lactic-co-glycolic acid (PLGA) are widely used [21]. PNPs are essential to modern imaging methods, helping to diagnose brain cancer through optical and magnetic resonance imaging [22]. Because they can pass through the blood-brain barrier, dendrimers, which have a three-dimensional core-shell polymer structure, offer better targeting. Dendrimers conjugated with tamoxifen serve as efficient drug carriers. Polyamidoamine is a commonly utilized dendrimer that is used for drug delivery. Surfactants are usually used in the manufacture of polymeric-based nanoparticles to lower the solution's surface tension. The polymer's building block, the monomer, is combined with the surfactant, and polymerization takes place, forming nanoparticles. Polymeric-based nanoparticles have some disadvantages despite their benefits, such as low stability, a propensity to assemble, and difficulties with production and scaling. Their usage in applications like medication delivery may be impacted by these restrictions. Protease-activated near-infrared fluorescent polymeric nanoparticles loaded with doxorubicin are useful for cancer imaging and treatment; similarly, 99mTc-PLA/PVA/Atezolizumab nanoparticles biodistribution evaluation is useful for identifying non-small cell lung cancer [23].

Dendrimers

Dendrimers, intricate artificial macromolecules with a well-defined three-dimensional structure, have garnered attention in nanomedicine due to their unique features. These nanoscale structures consist of a central core that is encircled by dendritic arms or branches. They have many functional groups. The molecular core, inner layer, and outer layer of dendrimers are the three layers that contribute to their multifunctional properties. This feature, along with their viscosity, solubility, and micellar qualities, renders dendrimers adaptable building blocks for sophisticated nanodevices intended for imaging and diagnosis. Dendrimers are globular structures with interior chambers that are usually smaller than 10 nm in size [24]. Dendrimers are used in many areas of the medical sciences and are essential to the advancement of the materials sciences. Dendrimers have been used in drug delivery, conjugation, encapsulation, nanocarriers, anticancer drugs, photodynamic therapy, gene delivery, medical theranostics, and biosensors in the field of medicine. Notably, because of their improved stability, water solubility, and decreased immunogenicity, dendrimers show promise in the administration of anticancer drugs. Furthermore, dendrimers have the ability to obstruct lymphatic drainage, cause hypervascularization, and increase the permeability of cancer cells. All of which are beneficial for passive targeting. These features aid in the targeted build-up of medication molecules in tumor tissues, improving the effectiveness of cancer therapy.

Divergent synthesis is the method used to create dendrimers, in which monomers are progressively added to a central core to create a highly branching structure. Customizing the size, shape, and reactivity of dendrimers is possible through the alteration of their monomers. The properties of dendrimers can be tailored to meet specific needs by varying the number of added monomers, reaction parameters, and monomer types. The synthesis process necessitates meticulous control over reaction conditions. Dendrimers are useful for targeted drug administration because of their special qualities, which include excellent stability, resistance to degradation, and solubility.

Exosomes and Other Emerging Platforms

Over time, the understanding of extracellular vehicles, or EVs, has changed dramatically. Previously regarded as artifacts or cellular debris, they are now understood to be significant players in several biological processes, serving a variety of purposes and facilitating intercellular communication [25]. Prokaryotic and eukaryotic cells both release EVs via mechanisms that have been preserved throughout evolution. EVs are categorized according to their size, origin cellular compartment, and location within or outside of cells [26]. It has been possible to identify prominent subtypes like tolerosomes, prostasomes, shedding vesicles, apoptotic bodies, exosomes, and prominosomes [27]. This classification highlights the variety of EVs and their roles in various physiological processes, as well as their importance as vital components of intercellular communication. The properties of exosomes, such as their nano size, non-toxicity, biocompatibility, low immunogenicity, and capacity to target with organotropism, make them advantageous for drug administration [28]. Exosomes can transport both hydrophilic and lipophilic molecules, making them similar in size to tiny unilamellar liposomes. Nonetheless, their organotropism and homing capacity are justified by the peculiar composition of their membrane. Organotropism is demonstrated by cancer-derived exosomes, which show a preference for merging with their parent cells [29]. The potential for overcoming the drawbacks of earlier drug delivery methods is considerable when exosomes are employed as a natural carrier platform for drug administration. Exosomes and their constituent parts, which come from biological systems, are readily digested and eliminated once the delivery process is finished. Furthermore, exosomes produce a less immunological reaction than cell treatments, which lowers the possibility of recipient rejection. Moreover, because exosomes are easily ingested and eliminated through the urine and bloodstream, it is thought that they have little tumorigenicity. Exosomes have been shown in studies to be able to control apoptosis, provide cytoprotection, and encourage angiogenesis. Notably, exosomes exhibit extraordinary ability for delivering difficult substances like proteins and RNAs and for bridging barriers, especially the blood-brain barrier (BBB).

The development of standardized, scalable, and economically viable Good Manufacturing Practice (GMP) processes for the extraction and purification of exosomes poses challenges [30]. Bioinspired exosome-like vesicles have evolved as a substitute for naturally derived exosomes to get around technical problems [31]. Exosome and liposome membranes combine to form hybrid exosomes, one type of artificial exosome-mimetic system [28]. By serially extruding a parent cell suspension through membranes with decreasing pore diameters, artificial exosome-mimetic systems can be produced. To treat cancer, a variety of medications, such as doxorubicin [32], paclitaxel [33], gemcitabine [34], aspirin [35], imperialine [35], miRNA [36], mRNA molecules [37], tumor necrosis factor-α [37], and recombinant methioninase [38], have been loaded into vesicles. Exosomes and exosome-mimetic systems are being actively developed and optimized by numerous research groups as drug-loading, surface functionalization, vesicle isolation, purification, and nanocarriers for the treatment of cancer. Exosomes have great potential for diagnostics applications beyond medication administration. They transport biomolecules from the cells that gave rise to them, which may include markers of pathological states. They are therefore essential for the development of biomarkers in clinical diagnostics. Exosomes are known to include proteins and nucleic acids linked to several illnesses, such as cancer, neurological ailments, metabolic issues, infectious diseases, inflammatory conditions, and more, according to recent studies. Furthermore, exosomes may be readily extracted from readily accessible bodily fluids like blood and urine, which makes them good candidates for diagnostic use.

The dual purpose of exosomes in the field of diagnostics: they can be used as active diagnostic tools that are modified with chemicals or nanoparticles for imaging procedures, or they can be used as passive biomarkers for cancer detection and prognosis [39].

1. Passive diagnostic application:

   Tumor exosomes that arise naturally are used as indicators for cancer prognosis and diagnosis. Their high concentration in blood makes it simple to identify them in small amounts of frozen plasma or serum [40]. The tumor stage can be ascertained by analyzing the proteome and genomic profile of tumor exosomes, which includes mitochondrial RNA, miRNA, and mRNA [41]. Utilizing silicon quantum dots embellished with a CD63 aptamer, scientists have been able to bind CD63 produced on exosomes extracted from human breast cancer cells [42]. This generates a nanoprobe for super-resolution imaging, allowing trafficking investigations in live cells and exploring the function of exosomes in cancer metastasis. Immunofluorescent labeling of particular molecules expressed on exosomes enables simultaneous dual-color imaging in an exosome-based super-resolution microscopy system [43].
2. Active Diagnostic Application:

   Different imaging techniques, such as optical fluorescence, computed tomography (CT), positron emission tomography (PET), single photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI), can be used to alter exosomes with chemicals or nanoparticles that can be detected [44]. This method works especially well for brain tumors and other difficult-to-diagnose cancers. Exosomes can support a precision medicine strategy by helping with the early detection of cancer metastases and recurrences [43] (Fig. 3).

2.2 Properties and Advantages of Different Nanocarriers

Conventional cancer diagnosis techniques, such as PET, MRI, and surgery, are expensive. Unique benefits of nanoparticles include improved targeting ability, volume proportion, and surface area. Their soft surfaces effectively penetrate tissues and epithelium, increasing bioavailability and extending the half-life. Because of these characteristics, nanoparticles have great promise for a wide range of biomedical applications, especially in the identification and management of different diseases. Carbon nanotubes, dendrimers, graphene, fullerene, metallic, polymeric, metal oxide nanoparticles, quantum dots, and liposomes are examples of synthesized nanoparticles.

Liposomes

Lipid bilayers make up liposomes, which resemble cell membranes in composition.

Advantages

- Versatility: Capability to transport both lipophilic and hydrophilic medications.
- Biocompatibility: The body’s general ability to tolerate anything.

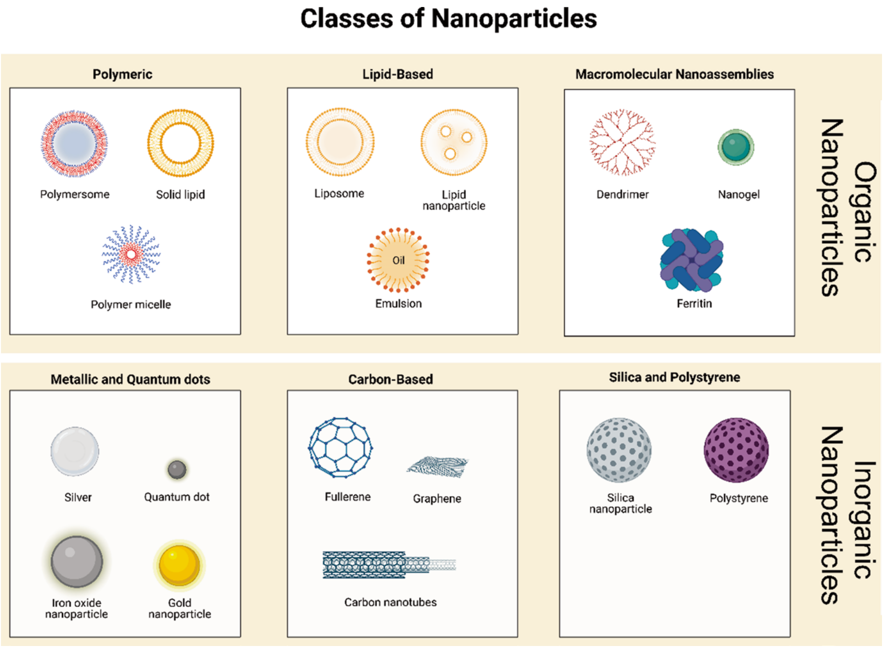
Classes of Nanoparticles
Polymeric
Polymersome
Solid lipid
Polymer micelle
Lipid-Based
Liposome
Lipid nanoparticle
Oil
Emulsion
Macromolecular Nanoassemblies
Dendrimer
Nanogel
Ferritin
Organic Nanoparticles
Metallic and Quantum dots
Silver
Quantum dot
Iron oxide nanoparticle
Gold nanoparticle
Carbon-Based
Fullerene
Graphene
Carbon nanotubes
Silica and Polystyrene
Silica nanoparticle
Polystyrene
Inorganic Nanoparticles

Fig. 3 Various classes of nanoparticles encompassing liposomes, polymeric nanoparticles, solid lipid nanoparticles, nanoemulsions, dendrimers, and micelles

- Targeting: Drug delivery can be specifically aimed at via surface changes.
- Encapsulation: Effective encapsulation of macromolecules as well as tiny compounds.

Polymeric Nanoparticles

Composed of biodegradable polymers such as chitosan or PLGA (poly-lactic-co-glycolic acid).

Advantages

- Controlled Release: Modifiable release kinetics to ensure long-term medication administration.
- Stability: Improved protection and stability for medications that are encapsulated.
- Biocompatibility: Usually, well-tolerated and safe.
- Surface Modification: Enables controlled release and targeted delivery.

Dendrimers

Tree-like, highly branching structures composed of repeating units.

Advantages

- Multifunctionality: The ability to target and load drugs into multiple functional groups.
- Size Control: Accurate control of dimensions and composition.
- Biocompatibility: Modifications can be made to increase biocompatibility.
- Drug Loading: High capacity for drug loading.

Micelles

Amphiphilic molecules in aqueous solutions form self-assembling structures.

Advantages

- Solubilization: Make hydrophobic drugs more soluble.
- Biocompatibility: Tolerable in most cases.
- Size Control: The product can be designed to meet precise size specifications.
- Versatility: Fits a range of medication kinds.

Metallic Nanoparticles (e.g., Gold, Silver)

Consists of nanoparticle-sized metallic components.

Advantages

- Surface Plasmon Resonance: Unique optical characteristics for diagnostic and therapeutic applications.
- Thermal Ablation: Capable of producing heat for focused thermal treatment.
- Drug Conjugation: To achieve targeted delivery, drugs can be conjugated or encapsulated.

Carbon Nanotubes

Carbon atoms are arranged into hollow cylinders with distinct structural characteristics.

Advantages

- High Surface Area: Enables a high loading capacity for drugs.
- Conductivity: Beneficial when imaging and therapy are coupled.
- Biocompatibility: Modifications can be made to increase biocompatibility.

Exosomes

Extracellular vesicles secreted by cells that occur naturally.

Advantages

- Immunogenicity: Lowers immunogenicity.
- The ability to target: Can be designed for targeted drug delivery.
- Biocompatibility: The body can tolerate it well.
- Payload Variety: Capable of transporting a wide range of payloads, such as medicines, proteins, and nucleic acids.

Silica Nanoparticles

Nanoparticles are composed of silica.

Advantages

- Tunable Pores: Drug loading may be possible with tunable porous materials.
- Stability: Stable and capable of resisting degradation.
- Surface Functionalization: Allows for surface modifications.

Quantum Dots

Semiconductor particles have quantum mechanical characteristics that are incredibly tiny. Light emission occurs according to particle size.

Advantages

- Fluorescent Properties: Because of its adjustable fluorescence, it is useful for imaging and diagnostics.
- High Quantum Yield: Efficient light emission.
- Multifunctionality: Capable of acting as both imaging agents and medication transporters at the same time.
- Targeting Capability: Drug delivery can be specifically targeted using surface modification.

Fullerene

Made up of carbon atoms grouped into a tube, ellipsoid, or hollow sphere having special optical and electrical qualities.

Advantages

- Antioxidant Properties: Anticancer activity may be aided by intrinsic antioxidant capacities.
- Versatility: Derivatives of fullerenes can be adapted for particular uses.
- Drug Carrier Potential: Possibility of drug encapsulation and delivery.

Graphene

Composed of a single layer with a hexagonal lattice arrangement of carbon atoms with a remarkably large surface area, offering lots of room for drug loading and having superior electrical conductivity.

Advantages

- High pharmacological Loading: Graphene's vast surface area allows it to hold a significant pharmacological payload.
- Biocompatibility: Improved biocompatibility is possible through functionalization.
- Versatility: Adaptable to different kinds of drugs and imaging agents.
- Benefits of conductivity: Good for combination imaging and therapy.

Selecting biomaterials for nanocarriers involves prioritizing safety, biocompatibility, biodegradability, and ease of assembly into nanostructures with desired characteristics.

Key considerations include:

- Safety: Ensuring that the selected biomaterials are safe for use in the body without causing adverse effects.
- Biocompatibility: To reduce the possibility of immunological responses or toxicity, biomaterials should be able to coexist with biological systems.
- Biodegradability: The ability of the nanocarrier to undergo degradation over time, releasing the drug payload and minimizing long-term effects on the body.
- Ease of Assembly: The biomaterials should be easily assembled into nanostructures with the desired properties.
- Delivery of Hydrophobic molecules: By efficiently moving hydrophobic molecules, nanocarriers can increase the solubility and bioavailability of these substances.
- Multifunctionality: The ability of nanocarriers to perform a variety of tasks, including medication administration, imaging, and targeting, increases their adaptability.
- Targeting Diseased Cells: Drug delivery can be more precisely delivered and off-target effects can be reduced by using nanocarriers that are specifically made to target diseased cells.
- Prolonged Systemic Circulation: Nanocarriers help medicines stay in the bloodstream for longer periods, which increases their therapeutic efficiency.
- Overcoming Drug Resistance: By employing combination therapy or targeted drug delivery, nanocarriers can assist in overcoming drug resistance.
- Encouraging Safety and Tolerability: By minimizing adverse effects on healthy tissues, the use of nanocarriers can improve the safety and tolerability of medications.

2.3 Design Considerations and Functionalities

Particulate materials with at least one dimension less than 100 nm are known as nanoparticles. Less than 100 nm-diameter nanoparticles are thought to be the best for using leaky vasculature to target tumors. Based on their overall shape, they can be divided into 0D, 1D, 2D, or 3D structures. The size of nanoparticles affects their physiochemical characteristics, including their optical properties. For instance, depending on their size, gold (Au), platinum (Pt), silver (Ag), and palladium (Pd) nanoparticles display distinctive colors (wine red, yellowish grey, black, and dark black, respectively). Changes in nanoparticle size, shape, aspect ratio, the thickness of the nanoshell, and concentration of gold cause variations in their color and characteristics. Three layers make up nanoparticles: Surface layer: Functionalized by metal ions, polymers, surfactants, or tiny molecules. Shell layer: Substance that differs chemically from the core. Core: The component of the nanoparticle that is in the middle.

NP adhesion is significantly influenced by surface functions. On nanorods, Janus coating improves adherence over uniform coating [45]. Polyethylene glycol (PEG) immobilization increases circulation half-life and decreases uptake by non-target cells by reducing protein adsorption and phagocytosis [46]. Phage display-based targeting and NP-aptamer bioconjugates are two of the techniques used in NP-based medication delivery [47]. Potential uses are demonstrated by the selective localization to organs made possible by certain peptides immobilized on NPs. Batch-to-batch variations result from post-synthesis surface alteration, which involves excess chemical reactants and reactions [47]. Higher reproducibility can be achieved by avoiding the requirement for post-particle alterations with pre-functionalized biomaterials that self-assemble into specific nanoparticles. The requirement for post-synthesis modification is eliminated by multicompartment micelles with a segregated hydrophobic core and a water-soluble shell. Amphiphilic biohybrid macromolecules and triblock copolymers are two examples of nanoscale architectures that demonstrate sophisticated production techniques [48]. Layer-by-layer self-assembly is used to create particles that replicate the architecture of RBCs, showcasing vital characteristics [49]. Particle lithography techniques are utilized to build drug carriers with the ability to target several functions and perform imaging functions. Large surface areas and high aspect ratio NPs enable the best possible introduction of several capabilities. Bimetallic nanorods with several segments are utilized in gene-delivery systems that have selective functionalization. Studies on controlled drug release employ mesoporous nanoparticles based on silica that have varying pore structures and morphological characteristics [50] (Fig. 4).

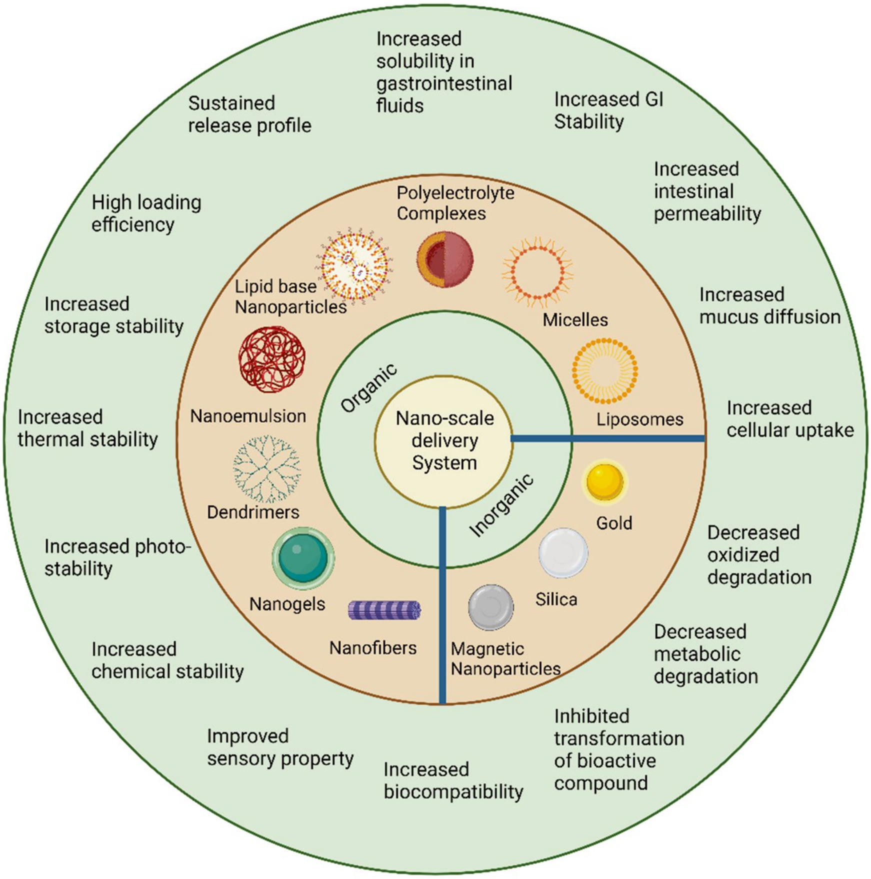
Increased solubility in gastrointestinal fluids
Sustained release profile
Increased GI Stability
Increased intestinal permeability
High loading efficiency
Increased mucus diffusion
Increased storage stability
Increased cellular uptake
Increased thermal stability
Decreased oxidized degradation
Increased photo-stability
Decreased metabolic degradation
Increased chemical stability
Inhibited transformation of bioactive compound
Improved sensory property
Increased biocompatibility
Polyelectrolyte Complexes
Lipid base Nanoparticles
Micelles
Liposomes
Nanoemulsion
Dendrimers
Nanogels
Nanofibers
Magnetic Nanoparticles
Silica
Gold
Organic
Inorganic
Nano-scale delivery System

Fig. 4 Summary of nanoparticle designs and their positive impacts on encapsulated bioactive substances

3 Targeting Strategies in Nanomedicine

3.1 Surface Modifications and Functionalization

Surface modifications are the process of modifying a nanomaterial’s surface characteristics without appreciably affecting its bulk characteristics. The stability, biocompatibility, dispersibility, and reactivity of nanoparticles can all be improved by these alterations. To give nanoparticles specified qualities or functions, functionalization entails adding particular functional groups or molecules to their surface.

1. Molecular Functionalization

   Covalent Functionalization: This is the process by which functional groups and the surface of nanoparticles create covalent bonds. It allows molecules to adhere to the surface of nanoparticles firmly and steadily. Covalent functionalization is achieved by methods like conjugation chemistry and chemisorption. Thiol groups (–SH), for instance, can be bonded to the surface of nanoparticles to offer a substrate for further functionalization [51].

   Non-Covalent Functionalization: Hydrophobic interactions, van der Waals forces, hydrogen bonding, electrostatic forces, and other weak interactions are examples of non-covalent interactions. Comparatively speaking, this process is milder and more straightforward than covalent functionalization. It is possible for molecules to reversibly connect to the surface of nanoparticles through non-covalent functionalization. For example, positively charged nanoparticles can be attached to negatively charged molecules by using electrostatic interactions [51].

2. Intrinsic Surface engineering

   Heteroatom Incorporation: Heteroatoms, or atoms other than carbon and hydrogen, are added to the surface of nanoparticles as part of intrinsic surface engineering. This has the potential to change a nanoparticle's reactivity, surface chemistry, and electrical structure. For instance, doping semiconductor nanoparticles with dopant atoms (nitrogen, boron) can change their electrical and optical characteristics, modifying their suitability for a range of uses including optoelectronics and photocatalysis.

   Engineering Defects: Defects engineering is the intentional introduction of flaws or vacancies into the nanoparticle crystal structure. The mechanical, catalytic, and surface characteristics of nanoparticles can all be impacted by these flaws. Defect control can be obtained during synthesis or by post-treatment procedures. For example, metal oxide nanoparticles can be made more catalytically active for oxygen reduction processes in fuel cells by adding oxygen vacancies.

3.2 Ligand Conjugation for Target-Specific Binding

In nanotechnology, ligand conjugation is essential for accomplishing target-specific binding, especially in biological applications including drug administration, imaging, and biosensing. Attaching particular molecules, referred to as ligands, to the surface of nanoparticles is known as ligand conjugation. These targeting moieties, which have an affinity for particular receptors or biomarkers found on the target cells or tissues, can be antibodies, peptides, aptamers, small molecules, or other types of ligands. Researchers can create nanomaterials to selectively bind to target cells, tissues, or molecules by conjugating ligands onto the surface of nanoparticles.

The specificity and effectiveness of certain applications, such as medication delivery, imaging, and diagnostics, are improved by this targeted binding.

Ligand Types

Antibodies: To precisely identify and bind to antigens on target cells, monoclonal or polyclonal antibodies can be attached to nanoparticles. This method is frequently applied to cancer treatment and tailored medication distribution.

Peptides: For targeted administration or imaging applications, short peptide sequences that have a high affinity for receptors or biomarkers can be attached to nanoparticles.

Short single-stranded DNA or RNA sequences known as “aptamers” have a high degree of specificity when it comes to binding to target molecules. Aptamers and nanoparticles can be coupled to deliver or detect proteins with precision.

Small Molecules: To achieve targeted delivery or imaging of sick tissues, small molecules, such as medications or imaging agents, can be coupled to nanoparticles.

Covalent reactions are the most common conjugation chemistries for nanoparticles. These include amine reactive groups (creating amide or amidine bonds), sulfhydryl reactive groups (making thioester, disulfide, or gold-thiol links), and carbonyl reactive groups (producing hydrazone or oxime bonds). Chemistry can also generate amide bonds or triazole rings. Furthermore, because the streptavidin-biotin contact is almost irreversible, it is a non-covalent interaction that is frequently used for nanoparticle conjugation. It has an incredibly strong binding affinity (Kd of $10^{-14} - 10^{-15}$ M) [52].

To improve the performance of nanoparticles, chimeric and multifunctional targeting systems have been developed. Chimeric targeting mixes ligands from different classes, whereas multifunctional targeting uses different ligands from the same class with distinct targets. These methods seek to enhance target specificity, cell uptake, and the application of various targeting techniques. Recent instances comprise the anti-EGFR and MOV18 anti-folate receptor α antibody-based nanoparticles developed by Bhattacharyya and team [53], and the Anx and RGD peptide-based nanoparticles developed by Kluza and team synthesized multifunctional and chimeric nanoparticles using three targeting ligands: peptide RGD, DNA aptamer AS1411, and DNA aptamer TTA1 [54].

3.3 Stimuli-Responsive Drug Release Systems

In nanotechnology, stimulus-responsive drug-release systems are novel drug-delivery devices that release therapeutic compounds in reaction to particular stimuli. By providing exact control over drug delivery, these devices maximize treatment efficacy and reduce side effects. These systems release drugs at the intended location by taking advantage of environmental stimuli including pH, temperature, light, enzymes, or magnetic fields.

1. Endogenous Stimuli-Responsive Systems

Redox-Responsive Mechanisms

Redox Potential in Tumour Cells: Compared to other cells, tumor cells have a higher redox potential due to their increased glutathione (GSH) levels [55].

GSH-Responsive Assemblies: These systems release therapeutic ingredients by using disulfide bonds that can be destabilized by GSH [56].

Hydrophobic Polymers: Sensitive to oxidative conditions, polymers such as polypropylene sulfide are appropriate for drug delivery during disease conditions [57]. As an example, disulfide-linked nanocarriers may find use in cancer treatment [58].

pH Stimuli-Responsive Systems

Microenvironment of Tumors pH: pH-sensitive nanoparticles undergo alterations due to the acidic pH of the tumor microenvironment, which results in regulated medication release.

Materials used: pH stimuli-responsive nanoparticles can be made from both organic and inorganic materials; however dendritic polymers are more frequently utilized because of their malleable nature [57]. Examples include the development of pH-sensitive nanoparticles containing anticancer medications like doxorubicin and curcumin, which exhibit faster drug release in acidic conditions and hence improve the effectiveness of cancer treatment [57].

Enzyme Stimuli-Responsive Systems

Enzyme-Specific Degradation: When enzymes unique to the target site break down nanomaterials having enzyme-responsive characteristics, the therapeutic payload is released [59].

Advantages: Often use proteases and phospholipases as triggers, provide biorecognition, sensitivity, and selectivity. Examples: Systems for targeted drug delivery using polymers or liposomes that are sensitive to enzymes have been created, and these systems show potential for use in imaging and cancer treatment applications.

microRNA Responsive System

Challenges: Delivering microRNA (miRNA) for therapeutic reasons presents a number of difficulties, including effects that are off-target and stable [60].

Delivery Assisted by Nanoparticles: By improving targeting and preventing miRNA degradation, nanoparticles increase the effectiveness of therapeutic interventions.

Examples: To carry miRNA, nanocarriers like gold and hyaluronic acid-based nanoparticles have been used. These nanocarriers have the potential to be used in combination treatments and gene regulation to treat cancer [61].

2. Exogenous Stimuli-Responsive Systems

Thermo-Responsive Systems

Mechanism: Work by releasing medications in response to temperature fluctuations, capitalizing on the elevated temperatures found in diseased tissues.

Techniques: They use burst release, which can be caused by external heat sources such as photothermal agents or at high temperatures.

Photo/Light-Responsive Systems

Mechanism: Drug release is triggered by certain light wavelengths in light-responsive systems; near-infrared (NIR) light is a potential wavelength due to its deep tissue penetration [62].

Techniques: For regulated drug release, two-photon absorption (TPA), upconverting nanoparticles (UCNP), and the photothermal effect are used. For example, drugs were combined with polycaprolactone (N-isopropyl acrylamide) (PNIPAM) coupled with carbon nanomaterials to treat tumors [63].

Magnetic-Responsive Systems

Mechanism: The mechanism of magnetic-responsive systems involves the release of medicines by directed targeting or hyperthermia, which is triggered by magnetic fields [64].

Applications: Magnetic chitosan hydrogels and superparamagnetic iron oxide nanoparticles (SPIONs) are being investigated for medication delivery and tumor inhibition.

Ultrasound-Responsive Systems

Mechanism: Ultrasound-responsive systems release drugs using the thermal and mechanical effects that are produced by ultrasonic waves.

Applications: To distribute drugs and accumulate in tumors, mesoporous silica nanoparticles (MSNPs) and PLGA nanoparticle aggregates react to ultrasonic stimulation [65].

Electric Field-Responsive Systems

Mechanism: The mechanism of electric field-responsive systems involves influencing the permeability of cellular membranes to release medicines, which has the potential to aid in tissue restoration and wound healing.

Applications: In reaction to applied electric potential, strategies entail the degradation of conducting polymers to release pharmaceuticals into the surrounding environment [66].

Dual Response Systems

Together with the single stimulus-responsive nanoparticles mentioned above, smart-responsive drug-delivery systems may be taken into consideration. Combined triggers that use magnetic fields and pH, temperature and redox potential, temperature and enzyme presence, temperature and pH, redox potential, and pH combined with temperature and redox potential [67].

Temperature and pH Dual-Stimuli-Responsive Systems

Temperature and pH dual-responsive systems take advantage of the particular circumstances found in tumor tissues to provide exact medication release. pH-responsive materials and polymers such as poly (N-isopropyl acrylamide) (pNIPAAm) can be combined to provide regulated medication release in response to variations in pH and temperature [68].

pH and Redox Potential Dual-Stimuli-Responsive Systems

Dual-responsive systems with pH and redox potential can effectively treat cancer by taking advantage of the conditions that have changed in cancer cells. Drug delivery efficiency is increased by redox-sensitive polymers or disulfide-bonded nanoparticles that release medications in response to pH and redox potential changes [69].

Dual-Stimuli-Responsive Systems

Dual-responsive systems use both endogenous and exogenous cues to integrate light and redox potential for tailored medication release. Smart drug release is demonstrated by NIR light and GSH-responsive nanoparticles, offering regulated drug delivery for cancer treatment [70].

Magnetic Field-Responsive Delivery System

For regulated medication release, magnetic nanoparticles are combined with materials that respond to pH or redox potential in magnetic field-responsive devices. Targeted drug delivery is made possible by pH-responsive and magnetic nanocomposites, which have potential medical uses [71].

Multi-responsive Systems

There is a growing interest in systems that are responsive to many stimuli. Multi-responsive systems are those that incorporate more than two sensitivities in a single matrix, such as temperature, redox potential, pH, magnetic fields, and temperature [72]. Because these devices function as intelligent carriers that release their payload when needed, they are essential to the field of nanomedicine [73].

Magnetic Field, Temperature, and Redox Potential Multifunctionality

In the tumor microenvironment, multi-responsive nanocarriers with integrated magnetic field, temperature, and redox potential stimuli provide improved drug release capabilities. Significant drug loading capacity and encapsulation efficiency are demonstrated by magnetic mesoporous silica nanoparticles (MMSNPs) with iron oxide cores. Up to 98% of the medicine is released by these nanoparticles in 24 hours in response to decreasing conditions, localized heating, and alternating magnetic fields [74].

pH, Light, and Enzyme Multifunctionality

Tumor detection and targeted drug release are made possible by the creation of nano-drug delivery devices with multifunctional properties such as pH, light, and enzyme responsiveness. Hyaluronidase (HA)-coated copper sulfide nanoparticles (CuS NPs) loaded with DOX exhibit regulated drug release that is induced by pH variations, exposure to near-infrared radiation, and enzymatic breakdown of HA [75]. These systems have the potential for using chemotherapy and photothermal therapy together to treat tumors.

4 Applications Across Disease Domains

4.1 Oncology

Targeted Chemotherapy

The National Nanotechnology Initiative (NNI), whose applications to drug delivery and discovery are referred to as nanomedicine, was launched by the US government in 2000. Since its start, nanomedicine has primarily focused on tumor-targeted drug delivery [76]. One promising field of nanotechnology for cancer diagnostics is targeted nanoparticles. Because these nanoparticles are made precisely to stick to cancer cells, medical practitioners can easily identify and target them [77]. Early organ tumor detection is made possible by nanotechnology, which solves the problem of late-stage diagnosis. The detection of cancer biomarkers in extracellular environments and *in vivo* bioimaging techniques is made easier by the sensitive and precise multiplexed measurement capabilities that nanotechnology provides [78]. Targeted nanoparticles may aid in more precise early identification, facilitating quicker diagnosis and improved tracking of cancer development. Biosensors are tiny devices that use nanotechnology to identify particular biomarkers in a patient's blood or other body fluids. Biosensors may help with early-stage detection and may be suggestive of malignancy. Despite the rise in popularity of notable nanomedicine formulations like Mylotarg®, Doxil®, and Abraxane®, the results have not been as good as expected. Based on the "enhanced permeability and retention" (EPR) effect, there have been few advances in tumor-targeted drug delivery, and there haven't been many newly approved anticancer formulations. Research on nanomedicine has produced important byproducts besides tumor targeting. Because of their small size, nanoformulation improve the kinetics of dissolution by making poorly soluble medicines more soluble in water. For instance, Rapamune® was the first nanocrystal formulation that the FDA approved in 2000 [79].

There are several interesting uses for nanoparticles specifically, quantum dots in cancer, particularly in imaging. Because of their quantum confinement, quantum dots have special features that enable size-tunable light emission. When combined with other imaging modalities such as magnetic resonance imaging (MRI), they can provide outstanding nanoscale imaging capabilities. Compared to conventional contrast agents like organic dyes, quantum dots have the advantages of being brighter and only requiring one light source for excitation. Imaging with more contrast is made possible by this feature. Furthermore, compared to organic dyes, quantum dots may be less expensive, which makes them a desirable choice for medical imaging. One significant issue with quantum dots is their composition-many of the compounds used to make them can be toxic. For example, certain quantum dots include potentially harmful heavy metals, such as cadmium. The clinical translation of quantum dots in medical applications requires addressing the toxicity issue. Quantum dots have a lot of potential applications in oncology imaging, however there are obstacles to be addressed. To fully utilize nanoparticles in medical applications while maintaining patient safety, more research is needed into safer materials and better production techniques.

One important characteristic of nanoparticles that increases their functionality and adaptability is their high surface area-to-volume ratio. This unique property allows various functional groups to be attached to the nanoparticles, enabling them to selectively bind to specific cells, such as tumor cells. The 10–100 nm size of nanoparticles is useful in the treatment of cancer. Because tumors frequently lack a functional lymphatic drainage system, nanoparticles are more likely to concentrate around tumor locations due to leaking vasculature. It is named enhanced permeability and retention, or EPR. With the help of the EPR effect, medicinal drugs can be delivered to the tumor specifically, potentially reducing harm to the surrounding healthy tissues. When it comes to treating cancer, nanomedicines have the potential to overcome issues including medication solubility, instability, and resistance [1, 80].

One intriguing approach for upcoming cancer treatments is the use of multifunctional nanoparticles. These nanoparticles provide a complete approach to cancer therapy, as they may be engineered to detect, image, and treat tumors. Other cancer treatment options include enhanced tissue imaging, understanding tumor microenvironments, and adjusting the release of nanoparticle-bound drugs. For instance, with Kanzius RF therapy, cancer cells are coated with small nanoparticles, which are then selectively heated by radio waves to fry the tumor while causing the least amount of harm to surrounding healthy tissue [81].

The combination of magnetic drug delivery technology and nanotechnology highlights their novel contributions to drug delivery and possible influence on cancer therapy. Using an external magnetic field, the magnetic drug delivery technique directs drug-loaded nanoparticles to a specific site [82]. By applying an external magnetic field, magnetic nanoparticles can be functionalized with medications and steered toward specific regions, such as tumors. More accurate medication delivery is made possible by this technology, which also minimizes systemic exposure and the negative effects of traditional drug delivery techniques [83]. By preventing the drug payload from degrading, nanoparticles enhance stability and extend the duration of drug release. Because of its versatility, the combination of nanotechnology and magnetic drug delivery technology has promise in several medical fields. By delivering chemotherapeutic medications straight to tumors, magnetic nanoparticles can help treat cancer by raising drug concentration at the tumor site and lowering toxicity in surrounding healthy tissues. By applying external magnetic fields, neuroactive drug-loaded nanoparticles can be directed to particular brain areas. The promise for transforming drug delivery through improved targeting, decreased side effects, and improved treatment results exists with the combination of nanotechnology and magnetic drug delivery technologies.

Additionally, nanowires are essential for the diagnosis of cancer. Nanowire-based sensor test chips are capable of identifying proteins and other indicators secreted by cancerous cells. With the use of this technique, a patient's little blood sample can be used for an early cancer diagnosis. Improving the prognosis and efficacy of cancer treatment requires early detection.

Effective drug encapsulation, successful drug delivery to the intended site of the body, and controlled drug release are all necessary for the use of nanotechnology in drug delivery for cancer therapy. Some instances of how different nanostructures are applied in cancer treatment are as follows:

Gold-Coated Nanoshells:

1. Dimensions and Coating: Gold-coated nanoshells measuring 120 nm in diameter.
2. Targeting: Attaching peptides or antibodies to the surface of the nanoshell so they can bind specifically to malignant cells.
3. Therapeutic Mechanism: An infrared laser is used to heat the gold and kill cancer cells in the tumor area. Mouse models have been used to illustrate this strategy.

Cadmium Selenide Quantum Dots:

1. Size: Cadmium selenide quantum dots have sizes ranging from 2 to 9.5 nm.
2. Detection: Quantum dots generate fluorescence when exposed to ultraviolet light, which helps identify cancerous tumors.
3. Application: By injecting these quantum dots into a cancerous tumor, doctors can make the tumor visible under UV light, which will make surgery simpler.

Using Nanoparticles for Photodynamic Therapy:

1. Size: Nanoparticles implanted into the tumor.
2. Illumination: The nanoparticles are activated by external light irradiation.
3. Therapeutic Mechanism: When light is absorbed by nanoparticles, high-energy oxygen molecules are produced. These molecules chemically react with tumor cells, destroying them without endangering healthy cells. This non-invasive method has grown in significance for treating tumors.

Different Nanosystems for Treating Cancer:

1. Carbon Nanotubes: Used in the identification of protein biomarkers and DNA mutations. These nanoparticles can be made functional by adding ligands or antibodies that bind to the receptors or proteins that cancer cell surfaces over-express [84].
2. Dendrimers: Used as image contrast agents and for controlled-release medication administration.
3. Nanocrystals: Used to identify cancer markers and improve the formulation of poorly soluble medications.
4. Nanoparticles: Used as reporters of apoptosis and angiogenesis, targeted drug delivery, MRI and ultrasound image contrast, and permeation enhancement.
5. Nano Shells: They are useful for deep tissue thermal ablation and tumor-specific imaging.
6. Nanowires: Beneficial for detecting gene expression, DNA mutations, and disease protein biomarkers.
7. Quantum dots: Used in optical imaging and detection because of their fluorescence and size.
8. Protocols for gene therapy and heat-induced ablation therapy are being combined with nanorobotics

Examples of Nanoparticle-Based Targeted Chemotherapy:

1. Doxil (Liposomal Doxorubicin): Doxorubicin-containing liposomal nanoparticles are used to treat Kaposi's sarcoma and breast cancer.
2. Abraxane, also known as albumin-bound paclitaxel, is a paclitaxel nanoparticle formulation used to treat lung and breast cancer, among other malignancies.
3. Albumin-bound rapamycin Nanoparticles: Used to treat advanced pancreatic cancer.
4. HER2-Targeted Nanoparticles: As a treatment for breast cancer, these nanoparticles are functionalized with antibodies that target HER2 receptors.
5. Polymeric nanoparticles: Targeted distribution of different chemotherapeutic medicines using customizable polymeric nanoparticles (Table 1).

Theranostics in Cancer Treatment

A new field called "cancer theranostics" combines diagnostic and therapeutic potential in one method. The words "therapy" and "diagnostics" are combined to form the phrase "theranostics." By combining therapy and diagnostics, cancer theranostics aims to deliver accurate and personalized treatment by customizing medicinal therapies for each patient according to the unique features of their malignancy. This multifunctional strategy seeks to reduce treatment-related risks and expenses while optimizing the efficacy of therapeutic interventions. Several important factors and difficulties are related to the creation of efficient theranostic systems [85]:

1. Biomarkers Selection: When utilized as imaging or therapeutic biomarkers, biomarkers need to have two essential properties:

   Overexpression in Cancer Cells: In order to facilitate precise targeting, the biomarker must be strongly expressed in malignant cells.
   Absence in Healthy Cells: In order to prevent off-target effects, it should be either absent or very little expressed in healthy cells.

2. Regenerable Ligand: For in vivo testing, ligands utilized for targeting should have a high degree of repeatability. For consistent and dependable theranostic results, ligand performance must be consistent.
3. Properties of Nanoparticles:

   Biocompatibility: In order to prevent damage to biological systems, nanoparticles need to be biocompatible.
   Biodegradability: To reduce any possible long-term impacts and to make it easier for the body to eliminate the nanoparticles, biodegradable nanoparticles are preferred.
   High Loading Capacity: The effectiveness of nanoparticles is increased by their capacity to hold a large concentration of therapeutic substances.
   Controlled Release: When nanoparticles reach the malignant site, they should show a profile of controlled release of therapeutic components.

Table 1 Trials using nanocarriers in clinical settings to treat cancer

Types of cancer	Phase	Interventions	Study title	Outcome	NCT ID
Breast cancer	Phase 2	Drug: gemcitabine, drug: Paclitaxel protein-bound particles for injectable suspension (albumin-bound)	ABI-007 (nab-paclitaxel) and gemcitabine in treating women with metastatic breast cancer	A percentage of patients with confirmed responses, progression-free survival, overall survival, and adverse event	NCT00110084
Breast cancer	Phase 2	Biological: bevacizumab, drug: carboplatin, drug: ABI-007	Carboplatin, paclitaxel, and bevacizumab in treating patients with locally recurrent or metastatic breast cancer	Overall survival, rate at end of treatment, and progression-free survival	NCT00654836
Lung cancer	Phase 2	Drug: carboplatin, drug: paclitaxel albumin-stabilized nanoparticle formulation, genetic: protein expression analysis, and 3 more…	Paclitaxel albumin-stabilized nanoparticle formulation and carboplatin in treating patients with stage IIIB, stage IV, or recurrent non-small cell lung cancer	Progression-free survival, overall survival, and incidence and intensity of adverse events assessed according to NCI CTCAE v. 3.0, overall response rate is referred to as complete or partial response as evaluated by RECIST version 1.0 criteria	NCT00729612
Breast cancer	Phase 2	Drug: abraxane, drug: bevacizumab, drug: carboplatin	Phase II Study with abraxane, bevacizumab, and carboplatin in triple negative metastatic breast cancer	The best clinical response, given as a percentage of participants, and the median proportion of progression-free survival, was estimated using Kaplan–Meier techniques. The research assessed successive plasma samples to detect the existence of particular angiogenic markers	NCT00479674

(continued)

Table 1 (continued)

Types of cancer	Phase	Interventions	Study title	Outcome	NCT ID
Pancreatic cancer	Phase 2	Drug: abraxane	Abraxane therapy in patients with pancreatic cancer who failed first-line gemcitabine therapy	The overall six-month survival rate the number of participants exhibiting a stable disease and the number of participants displaying a partial or complete response	NCT00691054
Breast cancer nos metastatic recurrent	Phase 3	Drug: NK105, drug: paclitaxel	A Phase III Study of NK105 in patients with breast cancer	Progression free survival, overall response rate, overall survival	NCT01644890

4. *In Vivo* Examination: Thorough *in vivo* testing is necessary to guarantee the safety, effectiveness, and repeatability of ligands and nanoparticles in practical settings.
5. Removal from the Human Body: The method by which theranostic agents including nanoparticles will be eliminated from the body throughout treatment needs to be taken into account.

Multifunctional and multimodal theranostics-based anticancer therapy is a novel approach that combines several parts of cancer treatment and diagnosis into one plan.

1. Multifunctional Nanomaterials: Due to their distinctive nanoscale features, nanomaterials, especially nanoparticles are incredibly adaptable. Within the field of theranostics, these nanoparticles can be created to fulfill several purposes, including targeted therapy, drug transport, and imaging.
2. Concurrent Diagnosis and Treatment: The main objective of theranostics therapy is to use these multifunctional nanomaterials for both concurrent cancer diagnosis and treatment. Through the integration of imaging properties into the nanoparticles, medical professionals may view malignant tissues, facilitating prompt diagnosis.
3. Multimodal Treatment Approaches: The word "multimodal" describes how many treatment methods are combined into one therapy. This can entail a combination of methods such as immunotherapy, radiation, and chemotherapy in the setting of theranostics. Because these methods are integrated into nanotechnology, a thorough and focused treatment plan is possible.

4. Targeted Drug Delivery: By delivering medicinal compounds to bodily sites, nanomaterials enable tailored drug delivery. This focused strategy minimizes harm to healthy tissues while increasing the treatment's effectiveness.
5. Real-Time Monitoring: By incorporating imaging agents with nanoparticles, medical professionals can monitor the therapy's effectiveness and distribution.
6. Personalized and Targeted Treatment: Personalized and targeted cancer treatment is made possible by theranostics' multifunctional and multimodal nature [86]. Better results may result from customizing the therapy for each patient based on diagnostic data and real-time monitoring.
7. Decreased adverse Effects: Theranostics may have fewer adverse effects than conventional cancer treatments. Reducing damage to healthy tissues, the tailored drug distribution and real-time monitoring make for a more concentrated and effective course of treatment [87].

For theranostics to effectively monitor targeted medication distribution and treatment response, nanoparticles must be able to generate a consistent and clear imaging signal. The following are some particular instances of how different classes of nanoparticles have been used in theranostic approaches:

Theranostics in Response to Magnetic Fields

An imaging technique for soft tissues that is non-invasive is magnetic resonance imaging (MRI). Magnesium oxide nanoparticles, which are frequently present in contrast agents, improve the precision of magnetic resonance images. Early detection of edema and infection has been demonstrated by iron oxide nanoparticles [88].

Gene Theranostics

In the field of gene theranostics, plasmid DNA (pDNA) containing a gene that causes genetic abnormalities in tumors is introduced into tumor cells. In order to transfer pDNA into tumor cells, synthetic and inorganic nanoparticles have been produced that function as gene vectors. For instance, gene delivery into liver cancer cells has been accomplished by the use of MRI-visible polymeric nanoparticles containing cell-targeting pullulans.

Theranostics That Responds to Radiation

Low doses of X-rays are used in radiation treatment (RT) to monitor the internal organs. High photoelectric absorbance metallic nanoparticles improve radiation dose delivery by increasing RT sensitivity.

Light-Sensitive Theranostics

Promising cancer therapeutics are light-mediated theranostics, such as photodynamic therapy (PDT) and photothermal therapy (PTT). Strategies mediated by near-infrared (NIR) light enable deep tissue penetration. PTT raises the temperature of target cells using light radiation, which causes apoptosis. PTT has made use of a variety of nanomaterials, such as semiconductors [82] and gold nanoparticles. In photodynamic therapy (PDT), photosensitizers are exposed to light, which produces reactive oxygen species (ROS) and causes cell death [89].

4.2 Neurology

Blood-Brain Barrier Penetration

Treatment of disorders affecting the central nervous system is significantly hampered by the blood-brain barrier (BBB). Adeno-associated virus vectors, virus-mimetic nanoparticles, erythrocyte-mimicking nano vehicles, cell-based delivery nanocarriers, cell-penetrating peptide-linked nano vehicles, extracellular vesicles, injectable hydrogels, tight junction modulators, and immunomodulators are a few of the drug delivery methods that have been developed to get past this obstacle. Successful Phase II clinical trials of paclitaxel trevatide (ANG1005) efficiently cross the blood-brain barrier through a protein linked to the low-density lipoprotein receptor [90]. The absence of appropriate blood-brain barrier models contributes to the ongoing difficulties in creating treatment regimens for conditions like multiple sclerosis, ischemic stroke, Parkinson's, Alzheimer's, and epilepsy [91]. Better models are essential for improving cancer treatment as well as for delivering drugs to the brain. A different method of directly delivering medication to the brain is through intranasal deposition and absorption on the respiratory and olfactory epithelia, which lowers systemic exposure and increases bioavailability [92]. For practical use, it is necessary to comprehend the underlying mechanisms and patterns of drug distribution in the brain, particularly for medications that become active only after binding to particular receptors in particular brain areas.

Neurodegenerative Disease Therapeutics

The realm of medicine, particularly the treatment of neurodegenerative diseases, has great promise for nanotechnology. The continuous degradation of the nervous system's structure and function is a hallmark of a group of diseases known as neurodegenerative disorders. This group includes disorders including Parkinson's, Alzheimer's, and Huntington's diseases. The critical role that nanotechnology plays in the treatment of neurodegenerative diseases, with a particular emphasis on the administration of medications to the central nervous system (CNS).

Nanocarriers for CNS Therapeutics:

> To administer medications to the central nervous system, a variety of nanocarriers, such as dendrimers, nano gels, nano-emulsions, liposomes, polymeric nanoparticles, solid lipid nanoparticles, and nano-suspensions, have been thoroughly investigated.

BBB Cargo:

> A major obstacle in the treatment of neurological disorders is the blood-brain barrier (BBB), which nanomedicines are made to pass. Transport processes like endocytosis and/or transcytosis are used to accomplish this.

Preclinical Achievement:

> Using nanomedicine, early experimental success has been attained in treating CNS disorders such as brain tumors, HIV encephalopathy, Alzheimer's disease, and acute ischemic stroke.

Advancing Nanomedicine:

> In order to further nanomedicine, increasing BBB permeability is a priority. For medicinal medicines to adequately reach the brain, advancements in this field are essential.

Minimizing Neurotoxicity:

> An additional crucial factor to take into account is the decrease in neurotoxicity linked to nanomedicines. This entails reducing any negative impact on the nervous system.

Parkinson's Disease (PD)

PD is the second most common neurodegenerative disease. It causes extreme difficulty with bodily motions and is characterized by neuroinflammatory responses. While current treatments try to increase patients' functional ability, they are unable to stop the neurodegenerative process from progressing. The goals of applied nanotechnology are CNS regeneration and neuroprotection. Research on basic nanotechnology is carried out in tandem with developments in cell biology, neuropathology, and neurophysiology. New technologies promising neuroprotection, permissive environments, and active signaling cues for directed axon development are being developed. The design, biometric modeling, and optimization of an intracranial nano-enabled scaffold device (NESD) are the main areas of research. In order to reduce the peripheral side effects of traditional Parkinson's disease medication, this device is designed to administer dopamine to the brain at particular sites. The goal of developing CNS nanomedicines in the future should be to use innovative targeting moieties to improve brain tissue selectivity and drug-trafficking performance.

Alzheimer's Disease

Alzheimer's disease is a neurological ailment that affects about 35 million people globally, making it a prevalent and significant condition. It has been determined that nanotechnology has important uses in the field of neurology, especially in treating Alzheimer's disease. By creating nanoparticulate entities, nanotechnology aids in the early detection and treatment of Alzheimer's disease. These substances are designed with a high selectivity for endothelial cells in the brain capillaries. The circulating forms of amyloid-β (Aβ), which are a hallmark of the pathogenesis of Alzheimer's disease, are highly favored by nanoparticles. The interaction with Aβ forms may induce a "sink effect," potentially leading to improvements in the Alzheimer's disease condition. NP-based bio-barcodes with ultra sensitivity, immunological sensors, and scanning tunneling microscopy techniques are cited as technologies that can identify particular Aβ types, such as Aβ1–40 and Aβ1–42.

4.3 Infectious Diseases

Antimicrobial Nanoparticles

As an alternative to antibiotics, the use of nanoparticles (NPs) to target bacteria is growing. When it comes to treating diseases caused by bacteria, nanotechnology might be especially helpful. As examples, consider the use of NPs in antibacterial coatings for medical materials and implantable devices to promote wound healing and prevent infection; in antibiotic delivery systems to treat illness; in bacterial detection systems to produce microbial diagnostics; and in antibacterial vaccines to prevent and control bacterial infections [93].

In industries like food, health, and agriculture, developing antimicrobial coatings, especially those based on nanotechnology holds promise for treating diseases linked to biofilms. Because they are resistant to antibiotics, biofilms, which account for 80% of microbial infections, present difficulties. Antibiotics, peptides, enzymes, metal-based coatings such as copper, silver, zinc, and gold, and nanoparticles are some of the antimicrobial strategies [94]. Through promoting osseointegration, decreasing bacterial adhesion, and averting drug-resistant infections, nanotechnology provides an efficient biofilm treatment. The continuous need for novel antimicrobial surface coatings is highlighted by the obstacles that still need to be overcome, such as worries about the evolution of antibiotic resistance and possible toxicity. The production of unique nanomaterials, especially metal and metal oxide nanoparticles with a high surface area-to-volume ratio that makes them efficient against microbial diseases, has been made possible by advances in nanotechnology [95].

Silver-Based Nanomaterials

There are several forms of silver-based nanomaterials, such as wires, plates, and particles. Because silver nanoparticles have so many uses, they are researched and used extensively [96]. Strong antibacterial effects against bacteria, viruses, and fungi are demonstrated by silver nanoparticles. Products including cosmetics, medical supplies, textile fabrics, dressings, coatings, and clinical applications all use silver nanoparticles. Silver nanoparticles are used in medicine to treat burns, diabetic wounds resistant to antibiotics, and persistent ulcers. Silver nanoparticles promote collagen deposition in wound therapy, speeding up wound healing and exhibiting anti-inflammatory properties. Silver nanoparticles, particularly those with sizes $\leq$10 nm, have the potential to be harmful to human cell types [97]. Researchers are looking into immobilizing silver nanoparticles on several support materials, including polymers, activated carbon, metal oxides, and graphene oxide, to address concerns about cytotoxicity. It is investigated how titanate nanotubes might be used to modify silver nanoparticles. Their physicochemical characteristics are changed as a result, improving their antibacterial, catalytic, and photocatalytic capabilities.

Gold-Based Nanomaterials

Nanorods, nano latches, nanoclusters of nanorods, and nanoshells are only a few of the several types of gold-based nanomaterials. By conjugating with other molecules or altering their nanostructure, gold-containing nanomaterials can have their characteristics optimized. Gold nanoparticles can be conjugated with various antimicrobial chemicals and antibodies to achieve antibacterial action. Strong antibacterial action against a variety of bacteria, including antibiotic-resistant types, is exhibited by antibiotic-conjugated gold nanoparticles. Antibacterial agents are made from a variety of metal nanoclusters, such as copper, gold, and silver. Superior capabilities in imaging, detection, and biological applications are exhibited by gold nanoclusters (AuNCs) derived from metal nanoclusters. Due to their ease of modification, polyvalent effects, photothermal stability, and high biocompatibility, AuNCs are frequently used in therapy as antimicrobial agents when coupled with various surface ligands [98]. Gold nanoparticles, or AuNPs, have been identified as promising candidates in pharmacology due to their anti-arthritic, anti-HIV, anti-malarial, and anti-angiogenesis properties. Gold nanoparticles are used in biomedical applications such as gene therapy, medication delivery, diagnosis, and catalyzing medicinal treatments.

Titanium Dioxide (TiO_2) Nanomaterials

Titanium dioxide (TiO_2) surface water separation mediated by ultraviolet (UV) light has been discovered, which is an interesting finding. TiO_2 nanoparticles have been thoroughly investigated for their potential uses in nanobiotechnology and nanomedicine. When compared to other nanoparticles, titanium dioxide's photocatalytic bactericidal action makes it very appealing. Because of their unique photocatalytic activity, physicochemical qualities, natural abundance, good thermal stability, low cost, non-toxicity, and high surface-area-to-volume ratio, TiO_2 nanoparticles are recommended for a variety of applications [99]. Because of their multiple uses, TiO_2 nanoparticles are used extensively in biological and environmental cleanup. TiO_2 nanoparticles are particularly intriguing for fighting multidrug-resistant strains because of their broad activity against fungus and bacterial pathogens. TiO_2-based nanocomposites are environmentally friendly since they have a non-contact biocidal action. As a result, the requirement to introduce potentially hazardous nanoparticles into the environment to achieve disinfecting effects is removed [99].

Zinc and Zinc Oxide Based-Nanomaterials

When compared to organic antimicrobials, inorganic antimicrobial agents have benefits such as improved stability, lesser toxicity, lower bacteria resistance, and better selectivity. In comparison to other inorganic antimicrobials, zinc oxide (ZnO) is emphasized for its benefits, which include better heat resistance, higher selectivity, lesser cytotoxicity, and higher stability. ZnO nanoparticles are highlighted because they have better qualities than the bulk material [100]. NP's larger contact surface area improves their interactions with cells, which maximizes their antimicrobial efficacy. Numerous investigations reveal that ZnO nanoparticles of smaller size have superior antibacterial activity in comparison to those of greater size, implying a size-dependent impact [101]. The use of ZnO in medical products is emphasized, especially because of its capacity to block UV rays. According to reports, ZnO nanoparticles exhibit strong antibacterial action against a variety of microorganisms. Due to their many characteristics and activities, ZnO nanoparticles are very interesting in biology and medicine. These include their capacity to speed up wound healing, their antidiabetic and chemiluminescent qualities, their cytostatic activity against cancer cells, their anti-inflammatory activity, and their use in bioimaging.

Copper and Copper Oxide-Based Nanomaterials

Nanomaterials based on copper and copper oxide are used in many different industries, including electronics, sensors, optics, solar cells, batteries, cleanup, and antimicrobials. The advantages of copper over metals like gold (Au) and silver (Ag) are underlined, including the fact that copper is more affordable and highly conductive. Surface oxide layers are present in the majority of artificially produced copper nanoparticles (Cu NPs), and CuO nanostructures are p-type semiconductors with a monoclinic structure and a high dielectric constant. The release of Cu^{2+} ions is thought to be the mechanism by which copper-based nanomaterials exhibit antibacterial activity. Bacterial mortality results from these ions' damage to bacterial cell membranes, entry into cells, and disruption of enzyme function. Because of their high surface-to-volume ratio, copper nanoparticles are recognized for their high reactivity as antibacterial materials. The antibacterial qualities of CuO nanoparticles (CuO NPs), stop the growth of bacteria, fungus, viruses, and algae. Copper-based compounds are frequently found in pesticide formulations and are employed in a variety of health-related disciplines due to their very effective biocidal capabilities. Because of their unique properties, CuO nanoparticles are used in many fields, including optics, gas sensors, solar cells, high-temperature superconductors, lubricants, catalysis, and medicine.

Application
By producing oxidative stress through reactive oxygen species (ROS), zero-valent iron (FeO) nanoparticles harm bacterial DNA and proteins in both gram-positive and gram-negative strains. Certain nanoparticles, such as copper (Cu) nanoparticles, have a preference for the carboxyl and amine groups found on the surface of bacteria. By creating cross-links between the nucleic acid strands, they could tamper with DNA molecules and cause the helical helix to become distorted. Certain nanoparticles, notably those made from Tulsi leaf extract and silver nanoparticles (nAg-Fs), show antibacterial activity against different strains and induce cell death using cytoplasmic leakage and perforation. When applied to *E. coli*, gold nanoparticles show

antibacterial action. CaO, MgO, and SiO_2-doped Fe_2O_3 are examples of metal oxide nanoparticles that have antibacterial activity. SiO_2-doped Fe_2O_3 NPs show bactericidal activity against particular bacterial strains, MgO nanoparticles suppress growth and generate reactive oxygen species (ROS), and CaO nanoparticles disrupt the integrity of bacterial cells. As demonstrated with gram-negative Pseudomonas aeruginosa, nanoparticles, such as biogenic selenium nanoparticles, prevent the formation of bacterial biofilms [102].

Targeted Antiviral Therapy

The host immune system is frequently weakened by viral infections, and relapses are common. Viruses can replicate within host cells and release their genetic material, which makes it difficult to create effective antiviral therapies. Many novel technologies have been studied recently for the diagnosis, prophylaxis, and treatment of viral infections. In particular, nanotechnology has emerged as a very promising strategy because of its ability to successfully treat viral illnesses and get beyond the drawbacks of conventional antiviral medications.

Three ways for site-specific medication administration have been made possible by advances in new nanomaterial synthesis: direct injection to a specific site, passive targeting, and active targeting. Drugs can be delivered to particular tissues or organs via passive targeting, which makes use of the intrinsic properties of nanoparticles. Drug distribution is facilitated by nanoparticles of different sizes and lipophilicity, especially after oral administration. Peyer's patches containing M cells are examples of surfaces with certain characteristics and small-sized nanoparticles that improve lymphatic tissue uptake. After being ingested, nanoparticles are delivered to lymphocytes through lymph channels, avoiding hepatic metabolism and building up in lymph nodes to enable lymphatic system targeting. Potential targeting of viral reservoirs in lymphatic tissue is made possible by this buildup. By using particles of the ideal size (10–100 nm) to travel lymphatic channels and spread across the interstitium, injectable systems using nanoparticles can target lymphatics. Compared to hydrophobic nanoparticles, hydrophilic nanoparticles may be removed from the interstitial region more quickly.

Drug delivery through active targeting involves a variety of techniques and mechanisms; surface modifications and stimuli-sensitive nanocarriers are crucial for accurate and regulated drug release in targeted areas [103]. Utilizing nanocarriers sensitive to internal (such as abnormal pH or temperature) or external (such as magnetic fields, temperature changes, or ultrasound) stimuli in conjunction with monoclonal antibodies specific to tissue or cell antigens for targeted medication delivery. The capacity of pH-sensitive nanocarriers to release medications in acidic environments, such as endosomes, where enveloped viruses shed their envelopes and infect cells, highlights the importance of these nanocarriers in drug delivery. Bringing up pH-sensitive carriers such as polymeric micelles, liposomes, and nanogels that, in reaction to pH changes, enhance intracellular drug release by becoming unstable in acidic environments (like lysosomes) and releasing their contents.

Describing how labeled nanocarriers can be guided by external stimuli like magnetic fields, temperature changes, and ultrasound. This is especially useful for magnetic drug targeting, which uses magnetic impulses to direct drug-containing microparticles to particular tissues so that they can accumulate in target organs. Differentiating between passive drug release based on the properties of the microparticle system and active drug release triggered by external stimuli like ultrasound.

Antiviral medications must be effectively delivered to specific locations within the body, and nanoparticles are essential for this. These nanoparticles are a mainstay in therapeutic applications because of their exact size and surface characteristics, which make them adept at targeted drug delivery. When administered orally, intravenously, or by inhalation, nanoparticles find their way to areas of injured or inflammatory tissue. In this case, the surface characteristics of the nanoparticles, which are frequently coated with cell-specific ligands like proteins, peptides, antibodies, or tiny chemical molecules allow drugs to move passively or actively from extracellular to intracellular fluid. Once within the targeted cells, nanoparticles open up channels in the cell membrane to allow the right medication to enter and trigger the right immunological response. The advantages of organic targeting agents are cost-effectiveness, chemical stability, and user-friendliness. Their affinity and specificity, though, may be restricted. Carbohydrates, for example, have been shown to glyco-target particular lectins yet interact weakly with cell surface receptors. Another technique for targeting ligands with nanoparticles is the use of antibodies, which are primarily manufactured monoclonality and target extracellular domains and related proteins. Notwithstanding their effectiveness, problems with conjugation, short circulation periods, lack of directionality, and antigen-binding still exist. Investigating smaller antibody fragments like FN3, Z domains, and DARPins for target-specific ligands is one way to try to overcome these difficulties. Peptides are more advantageous than antibody-based ligands because of their increased affinity, specificity, and lower size. The effectiveness of naturally occurring peptides in targeting systems across metallic nanoparticles, micelles, polymers, and dendrimers has been investigated. Examples of these peptides are EGF, cANF, Angiopep-2, and RGD. Peptides are more expensive than other ligand types because of their limitations, which include cytotoxicity, low oral bioavailability, allergic responses, and sensitivity to proteolysis. Moving on to the challenges with the antivirals that are available now, include problems with bioavailability, drug resistance or tolerance, short half-lives, frequent administration requirements that result in non-compliance, exorbitant pricing, and possible harmful interactions with other medications. These drawbacks have led to research into nanotechnology as a viable means of addressing these issues and boosting the effectiveness of antiviral medication.

When coupled with silver nanoparticles, graphene oxide (GO) and reduced graphene oxide (rGO) exhibit increased antiviral capabilities by causing damage to viral membranes and promoting the photodegradation of viruses [104]. With their minimal cytotoxicity and ability to block connections between viruses and cells via their surface functional groups, carbon dots, or C-dots, show promise against viruses such as HIV-1 and Herpes simplex virus type 1 [105]. Because they produce [106].

Even with their solubility issues, fullerenes are low-cytotoxic and have the potential to treat a number of viral infections, such as herpes simplex virus, respiratory syncytial virus, and hepatitis C virus. Antiviral therapy can benefit from tailored drug release patterns provided by cyclodextrin-based delivery systems, which provide choices for quick release in case of emergency and extended release to minimize dose frequency. While pH-dependent and site-specific releases target specific settings to ensure efficacy and flexibility in therapy, modified release mechanisms accommodate medicines with poor solubility. Micelles, which are composed of a hydrophobic core and a hydrophilic corona, provide a useful vehicle for sustained blood flow and regulated release of antiviral medications. One example of this is [107]. Modified micelles containing chitosan or methoxy poly (ethylene glycol) (mMPEG) improve drug delivery efficiency and streamline production procedures using polycaprolactone (PCL) as a foundation material [108]. This results in a non-toxic and affordable solution. Exosomes are naturally occurring vesicles released by cells that exhibit potential for the administration of antiviral drugs by expressing proteins triggered by interferon and preventing virus multiplication. Nevertheless, more in vivo research is required to verify their effectiveness in human bodies. Exosomes may be used to treat influenza since studies in mice have shown they can cause pulmonary inflammation, prevent viral invasion, and act as carriers of antiviral proteins. Lipid nanoparticles (LNPs) are a key component of lipid-polymer delivery systems, which hold great promise for mRNA therapies. LNPs augmented with PLGA have been shown to significantly increase transfection effectiveness. PLGA4 is the most improved PLGA among those under investigation in terms of zeta potential, encapsulation efficiency, and delivery efficiency, which makes it a better option for long-term mRNA release in nano vessels.

Solid lipid nanoparticles (SLNs) and liposomes present encouraging alternatives for antiviral medication delivery. Liposomes are able to transport high drug dosages while shielding them from deterioration due to their phospholipid bilayers. They can be adjusted using both passive and active loading strategies for longer release durations and increased stability. Their targeted drug delivery capabilities are improved when hydrophilic polymers are used to modify their surfaces. With a solid lipid matrix, SLNs offer regulated drug release and improved stability, much like liposomes. They enhance FDA-approved antiviral drugs' lymphatic system absorption, bioavailability, and drug permeability. SLNs, such as nanostructured lipid carriers, have a higher loading capacity and scalability than liposomes [109]. Though further study is required to fully realize their promise in molecular biology and nanomedicine, elastin-like polypeptides (ELPs) provide adaptable and intriguing prospects for antiviral applications, including as carriers for drug delivery and immune response control. Dendrimers' highly branched structure and surface functionalization make them an adaptable platform for antiviral medication delivery. They have been proven to be effective against a number of viruses, such as HIV, respiratory syncytial virus, and influenza. Furthermore, dendrimers demonstrate potential for treating newly developing viruses such as MERS-CoV, underscoring their importance in the fight against worldwide pandemics.

Nanoemulsions have benefits like improved absorption, targeted medication distribution, and greater bioavailability due to their small particle size and capacity to solubilize both hydrophilic and lipophilic medicines [110]. Furthermore, research using antiviral medications like Nevirapine shows that self-nano emulsifying drug delivery systems (SNEEDS) spontaneously generate lipid-based systems, enhancing drug solubility and bioavailability [111]. Nanogels are stable and multipurpose for the administration of antivirals because they have a high encapsulation efficiency and protect active chemicals well. The ability to release specific compounds in response to stimuli is demonstrated by pH-sensitive nanogels that have antiviral properties against HIV-1 infection. Comprising only the drugs and a small number of stabilizers, nanocrystals present a potentially effective way to increase drug solubility; their characteristics can be adjusted by temperature and stirring rate. Nanocrystals have been used in antiviral applications; one study found that structural alterations to modified Carbon-60 nanocrystals increased their antiviral activity against the λ-bacteriophage. With their high drug-loading capacity and cytocompatibility, metal-organic frameworks (MOFs) provide effective regulated drug delivery for antitumor and retroviral medications [112]. This makes them promising for therapeutic applications, particularly the treatment of cancer and viral infections. In addition to metal oxides like CuO, TiO_2, CeO_2, and SiO, gold and silver nanoparticles have also shown excellent efficacy against a wide range of viruses by engaging with viral proteins through Van Der Waal forces and Kazimir interactions. Though they have promised antiviral activities, worries about toxicity limit their prospective application. Nanobubbles, Nanofibers, Nanodiamonds, Nanotraps, and Nanorobots are considered future nanosystems for antiviral drug delivery.

5 Personalized Medicine and Nanomedicine Integration

5.1 Tailoring Treatments to Individual Patients

There are several places where personalized medicine and nanotechnology Integrate. First, the diagnostic field, where nanotechnology can be very helpful in pharmacogenetic testing, investigating the state of certain medication targets, and doing both *in vitro* and *in vivo* testing. The second is the therapeutic area, where a drug can be customized using nanomedicine to a specific target found for a specific disease in a particular patient [113]. In addition, the targeting power of nanomedicines allows for much higher dosages than the maximum tolerated dose of a non-formulated drug. Accordingly, the dosage can be adjusted to account for each patient's unique condition [114]. Lastly, the two key factors that influence a person's unique medication response are circumvented by nanomedicine. These factors are associated with the variations in cytochrome-P enzymes (CYP) and drug transporters among various groups. Drugs designed with nanomedicines may be able to elude enzymes that

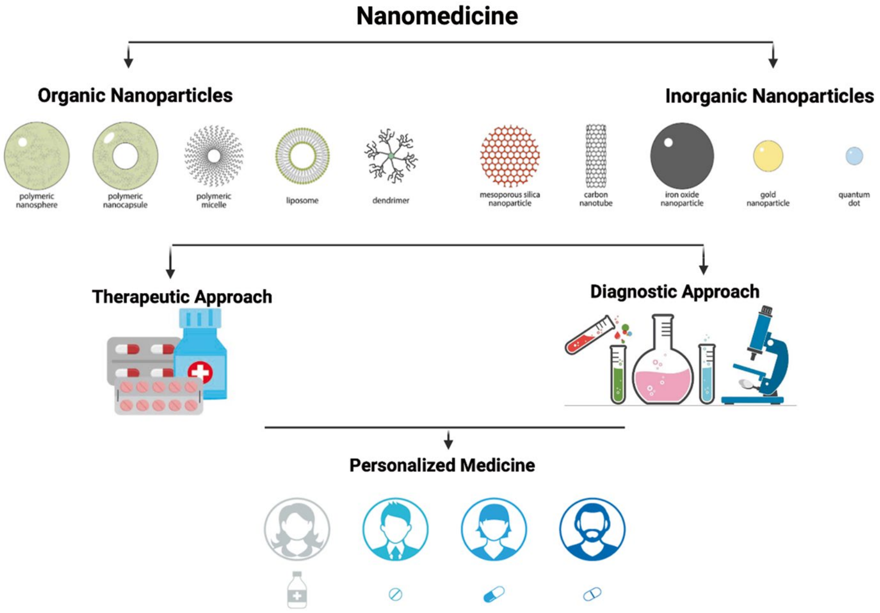
Nanomedicine
Organic Nanoparticles
Inorganic Nanoparticles
polymeric nanosphere
polymeric nanocapsule
polymeric micelle
liposome
dendrimer
mesoporous silica nanoparticle
carbon nanotube
iron oxide nanoparticle
gold nanoparticle
quantum dot
Therapeutic Approach
Diagnostic Approach
Personalized Medicine

Fig. 5 Integration of personalized medicine and nanomedicine for targeted therapeutics and enhanced patient care

break down their structure and enter cells through the endocytic process without the need for a transporter [115]. Nanomedicine can be utilized to develop medicines that can reduce the systemic effects that would otherwise be induced by conventional treatments like chemotherapy, as well as for targeted drug administration, imaging, diagnosis, cell identification, replacement, and repair [116]. By creating a dosage difference between the targeted site and the rest of the body, nanodrugs can maximize therapeutic effects in the targeted area while minimizing negative effects on the rest of the body when used to treat diseases [117]. Nanoparticles can be used as the carrier or vehicle for cancer therapy. They can encapsulate medicinal molecules or combine with them to generate a complex. It has been demonstrated in certain research that Nanoparticles can transport medications to malignant cells without harming healthy cells [118] (Fig. 5).

5.2 Role of Nanomedicine in Patient-Specific Therapeutics

According to several studies nanoparticles can be used as the carrier or vehicle for cancer therapy. They may contain medicinal drug molecules or combine with them to generate a complex. It has been demonstrated in certain research that Nanoparticles can transport medications to malignant cells without harming healthy cells [118]. In addition to being combined with radiolabeling material, Nanoparticles have significantly contributed to research on drug delivery tracking and imaging in both in vitro and in vivo conditions. Additionally, drug/macromolecule nanoencapsulation improves the therapeutic level, specificity, tolerance, and efficacy of the specific drugs. When interacting with the biological environment, nanoparticles can prevent the integrated medications from degrading too soon. Additionally, because of their nanoscale shape and structure, these small particles can aid the medicine that is contained inside of them to penetrate cells, increase retention duration, and enhance absorption [119]. Customized ways of cancer treatment are being developed with the help of nanomedicine. Personalized cancer treatment is being made possible by the current investigation of multifunctional and multiplex nanoparticles. Nanoparticles have a high affinity and specificity for malignant tumors when they are conjugated with bio-targeting ligands, such as peptides, monoclonal antibodies, or small compounds. These advancements offer prospects for the advancement of personalized oncology, wherein cancer identification, diagnosis, and treatment are customized to each individual's molecular profile.

Improvements in the nervous system's use of nanomedicines will enhance our knowledge of neurological conditions and drug development and delivery of medications for disorders with CNS [120]. Additional ways that nanotechnologies can aid in the advancement of personalized neurology include the development of logical treatments based on the pathomechanisms of neurological conditions. Using nanoparticles to deliver drugs to the brain directly is an essential step toward developing individualized treatments for neurological diseases [121]. Nanomedicines that can both diagnose pathology and treat it with tailored delivery systems are already having an impact on the future of cardiovascular diagnostics. Perfluorocarbon nanoparticles are used in cardiovascular illness to deliver drugs to specific targets while also serving as a tool for molecular imaging and disease visualization [122]. When it comes to coronary artery disease, molecular imaging uses diagnostic techniques that focus on biomarkers linked to the development of atherosclerotic lesions. Bioengineered nanoparticles have the potential to serve as carriers of therapeutic or diagnostic compounds in the treatment of coronary artery disease [123].

5.3 Advancements in Diagnostics and Therapeutics Integration

Nanoparticles can be used to combine diagnostics with therapeutics. The field of nanomedicine is expanding the capabilities of molecular diagnostics and enabling point-of-care diagnosis, therapeutics-diagnosis integration, and personalized medicine development [124]. Numerous nanoparticles have been applied to medical diagnosis. Magnetic nanoparticles, QDs, and gold nanoparticles are the most commonly used of them. Gold nanoparticles can be detected using a range of analytical methods, they are especially useful labels for sensors. Gold particles with a diameter of no more than 13 nm can have small bits of DNA linked to them. When a complementary target is present, only then do the gold nanoparticles assemble onto a sensor surface. The method may identify millions of distinct DNA sequences at once if a patterned sensor surface made of several DNA strands is applied. Inorganic fluorophores known as QDs have several benefits over fluorescent markers that are typically utilized in the industry. QDs allow for the integration of medicines and diagnostics, as well as multiplexed diagnostics. There are numerous uses for QDs in genotyping and molecular diagnostics. The two most significant applications of QDs are in the diagnosis of cancer and viruses, such as the rapid and precise identification of respiratory syncytial virus (RSV) [125].

MRI has been utilized in conjunction with superparamagnetic nanoparticles, which are 2–3 nm in size, to reveal small lymph node metastases that would otherwise go undetected. MRI imaging of brain tumors is improved by dextran-coated iron for the visualization of intracranial tumors for more than 24 hours. With a wide range of nanoparticle technologies, it is possible to modify the surface of nanoparticles to specifically bind a subset of biomarkers and isolate them for further investigation using highly sensitive proteomic testing [126].

DNA separation and biomarker identification can be performed quickly with the use of functional polymer-coated nanoparticles. The primary uses of cytogenetics have been used to characterize chromosomal abnormalities and explain their relationship to disease. The use of fluorescent in situ hybridization (FISH) in conjunction with traditional fluorescence microscopy to localize specific gene probes has reached its limit. The application of nanobiotechnology, such as quantum dot (QD) FISH and atomic force microscopy, has improved molecular cytogenetics. Chromosome probes and G-bands have provided local information that has been obtained by both atomic force microscopy and scanning near-field optical microscopy. In comparison to typical procedures, the final resolution permits a more accurate localization, and the scanning probe can collect minuscule amounts of chromosomal DNA. The integration of biochemical and nanomanipulation techniques, which allows for the nano dissection and nano extraction of chromosomal DNA, is a further focus of this method of research [127]. Some of the clinical applications of Nano diagnostics are to identify harmful germs at the point of treatment in a timely and accurate manner. The majority of conventional diagnostic techniques are limited by their lack of ultrasensitivity and their slow rate of result retrieval. Within 20 min, a single bacteria can be detected using a bio-conjugated nanoparticle-based bioassay for in situ pathogen quantification [128]. Using multicolor oligonucleotide-functionalized QDs as nanoprobes, a hybridization-detection approach has been used to detect single-molecule hybridization [129].

6 Challenges and Future Directions

Personalized medicine and nanomedicine are already prevalent in clinical settings, despite not being recognized as medical specialties. Both will interact, develop, and have a significant impact on how medical procedures are carried out in the future.

Enhancing healthcare through the application of new technology is the goal of personalized medicine. Of them, nanobiotechnology will be the most crucial in facilitating the combination of treatments and diagnostics, which is a fundamental aspect of personalized medicine. The number of nanomedicines being developed as therapeutics has increased quickly, but the clinical translation efficiency isn't up to the same level. This suggests and emphasizes various issues with nanomedicine that should be resolved before making them available for translational use. Clinical trials must be conducted as customized therapy methods to adapt to the rapidly evolving field of medicine. Early in the medication development process, multicenter collaboration is essential for patient enrollment in clinical trials. In addition, there are established methods in the field of drug research, such as regulatory approvals based on nonrandomized trials, tumor-agnostic approvals, and phase 1 expansion cohorts substituting phase 2 tests. However, there are challenges with patient accrual, unsolved regulatory matters, and the technological limits of molecular diagnostics that need to be addressed in clinical trials [130].

6.1 Scalability and Commercial Viability

The absence of guidelines and standards for safety, efficacy assessment, quality control, and manufacturing processes hinders the development of nanomedicines in general. Regulatory bodies like the USFDA and EMA set standards for nanomedicines to monitor them and offer suggestions but as of yet, no particular regulations have been developed to control their manufacturing or clinical translation. Various geographic locations have various approaches when it comes to applying nanotechnology. For example, a nanomedicine that is allowed in one country may not be approved in another [131].

6.2 Regulatory and Safety Considerations

Investigations are being conducted into the safety and regulatory aspects of nanomedicine, and the ethical and financial challenges of personalized medicine are also being addressed. Not all diseases can be treated with nanomedicine or personalized medicine, and a lot of areas where these treatments are both secure and affordable are being identified. The primary concerns surrounding nanomedicines pertain to their biological safety, affordability, and high-end, wide-range production. Similarly, legal, ethical, and social concerns present difficulties for precision medicine, which focuses on identifying and tailoring a person's DNA to treat a particular disease [132]. When given to the body, nanomedicines come into contact with the biological environment, but there are no defined safety evaluation parameters that can be used to assess their toxicity. Furthermore, when in systemic circulation, morphological and physiochemical traits may influence biodistribution and interact with biological membranes, endangering safety. Certain nanomedicine products may have problems, and these products further investigations might be necessary [133]. While there is concern about potential hazardous effects when using nanoparticles in vivo, this is not the case for in vitro diagnostics, which make up the majority of laboratory diagnostic procedures. The effects of nanoparticle manufacturing on the environment and the discharge of nanoparticles during that process are environmental concerns. In addition to naturally occurring nanoparticles in the environment, they are also being investigated. Regarding the fate of nanoparticles injected into living organisms, there are still a lot of unsolved concerns. These impacts will differ greatly due to the vast array of materials that are employed and the large variety and sizes of nanoparticles. Calcium and zinc ions, which can be harmful to cells, may be released from QDs that have been stabilized by adding fluorescent labels of calcium selenide or zinc sulfide [134]. Receiving informed permission can be a difficult procedure in a number of situations because patients undergoing drug testing as part of personalized treatment must be aware of any possible risks. Furthermore, the expense of developing a precision medication is quite high and will require funding for years with an alarming uncertainty of success, similar to the creation of nanomedicine. The instruments needed to create nanomedicine and sequence a person's DNA additionally add to costs. To reduce the possibility of failure and improve knowledge and understanding of a patient's particular conditions, it is important to understand the disease at the molecular and genetic level, interpret the genetic testing that the patient needs, and then design the medication.

6.3 Future Trends and Prospects

Nanotechnology-based measurement instruments that can quickly and affordably perform hundreds of measurements will be accessible within the next 10 years. The trend of biochip technology being reduced to the nanoscale will continue in the field of diagnostics in the future. Blood protein analysis will be the most often used clinical diagnostic application. The condition of most organs' health or sickness is reflected in the blood's systemic circulation. Thus, the identification of blood molecular fingerprints will offer an accurate evaluation of health and illness [135]. The application of nano-diagnostics has the potential to shorten test result wait times shortly. When patients arrive at the outpatient clinic or doctor's office, for instance, patients with STDs may be asked to produce urine samples; the results may be available by the time the patient sees the doctor. The prescription may then be delivered to patients right away, cutting down on the amount of time they have to wait for results. This would reduce patient anxiety and save overall costs. Nanobiotechnology will be important in the decade to come not only for diagnosis but also for the development of personalized medicine and the connection between diagnosis and treatment. Cancer diagnostics will be a significant further application area. It's often too late to cure cancer by the time it's discovered with the techniques that are currently available. In the future, nanorobotics may be used for both cancer treatment and early diagnosis. In individuals who do not show any overt signs of cancer, a nanodevice for simultaneous diagnosis and therapy might be implanted as a preventative strategy, and cancer surveillance could be carried out via external remote monitoring. This method of monitoring could identify cancer early on and provide the right kind of intervention. There would be a greater probability of recovery with early detection. Compared to biomarker detection in bodily fluid specimens, which can only be done rarely and is less reliable than continuous in vivo studies, such a device would have advantages [120].

1. *AI and Machine Learning in Nanomedicine*

In materials science, AI- and ML-assisted research is a relatively new space to explore. This can be observed in the quick development of data-driven nanomaterial design, the wide control over its interactions with living things, and the search and optimization of nanosystems. The development of multi-property predictive algorithms, genetic and generative models focused on nanomaterials, interpretable and general artificial intelligence, inverse nanomedicine design from the desired properties to the synthesis procedure, in silico preclinical studies, and digital twins' technology for biological and chemical systems modeling are some examples of the future directions of the approaches in this area [136].

2. *Theranostic Platforms and Next-Generation Applications*

The development of precise, highly effective medications that accumulate in lesions rather than in healthy non-target tissues is one of the top priorities for the next generation of nanotherapeutics. The number of publications in nanomedicine is increasing at a rapid pace, indicating the success of academic achievements in nanotherapeutic research. Numerous nanotherapeutics, such as polymeric, liposomal, nanocrystal, protein-based, and inorganic nanodrugs, have been approved for clinical use or are the subject of clinical trials. While they present many prospects, nanotherapeutics present more difficulties than conventional medications. Although traditional medications stay on the market, their use of nanotechnologies to lessen side effects and increase efficacy is growing. The widespread application of biotechnologies like gene therapy will be greatly enhanced by nanotherapeutics which play an essential route for improving conventional medications through nanotherapeutics.

7 Conclusion

With the use of a precise, tailored diagnosis based on an individual's genetic profile and focused treatment for that disease condition, a new field of research in personalized/precision medicine is emerging. It considers a patient's genetic, phenotypic, and environmental factors that may impact the safety and effectiveness of a specific medication, either individually or in a group of related individuals. To apply nanotechnology to personalized medicine, researchers have been merging several parts of the technology in recent years. Not only has nanomedicine made drug delivery possible, but it has also made molecular and genetic diagnosis possible [115]. Due to the benefits of nanotherapeutics in terms of pharmacokinetics, therapeutic efficacy, and safety, the nano-pharmaceutical industry has experienced significant growth and development. Numerous nanotherapeutics are being studied in clinical trials or have been authorized for use in medicine.

7.1 Recap of Nanomedicine's Impact on Precision Therapeutics

The most significant clinical uses of currently accessible nanotechnology are in the fields of infectious microorganism detection, cancer diagnostics, and biomarker identification. By enabling point-of-care diagnosis, the integration of diagnostics and therapeutics, and the development of personalized medicine, nanotechnologies hold the potential to overcome the limitations of current molecular diagnostics. The field of nanomedicine holds significant potential for advancements in the field of diagnosis and treatment techniques [124].

7.2 Vision for the Future of Personalized Medicine

Through the use of nanomedicine, personalized medicine can maximize therapeutic efficacy with a regulated drug release profile, improve bioavailability and compatibility, and increase binding affinity so that the drug reaches its intended target in the correct patient at the right time. Additionally, by comprehending a person's DNA, endpoint techniques for diagnosis and treatment can be designed. Promising outcomes have been shown using laboratory-produced nanoparticles, and it may only be a matter of time before customized medicine uses nanomedicine on a much larger scale. The three key features that will be required for future nanomedicine are intelligence (through AI-guided nanostructure design and nanorobot application), precision and personalization (through combining patient stratification and customized regimen selection), and multifunctionality (by integrating properties like diagnosis, imaging, and therapy). The fields of customized and nanomedicine have great potential to shape the medical landscape in the future. Nanomedicine can be the future of medicine and more advancement can be seen in this field in the near future.

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Nanomedicine in Cardiovascular Diseases: Diagnostic and Therapeutic Innovations for Better Health

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1 Introduction to Cardiovascular Diseases

Cardiovascular diseases are a group of disorders affecting the functioning of heart and blood vessels including angina, arrythmia, coronary heart disease, coronary artery disease, congenital heart disease, heart attack or myocardial infarction, deep vein thrombosis and pulmonary embolism [1]. Around 17.9 million people died from CVD in the year 2019, making it one of the deadliest diseases to mankind [2]. CVD accounted for only 10% of total deaths at the beginning of the twentieth century, which increased to 30% by the beginning of the twenty-first century and most cases are attributed to low-income developing countries [3]. CVD is a complex and multifactorial disease, with both genetic and lifestyle factors playing a role in its development. The risk factors for CVD are numerous and include unchangeable factors like age, gender, and genetics, as well as modifiable factors like poor diet, high blood pressure, high cholesterol, smoking, obesity, diabetes, dyslipidaemia, and physical inactivity (Fig. 1) [4, 5]. These risk factors can interact with each other and contribute to the progression of CVD. Understanding these risk factors is crucial for preventing and managing CVD. CVDs are chronic conditions that develop gradually over time and often do not show symptoms for a long period [6]. Atherosclerosis, coronary

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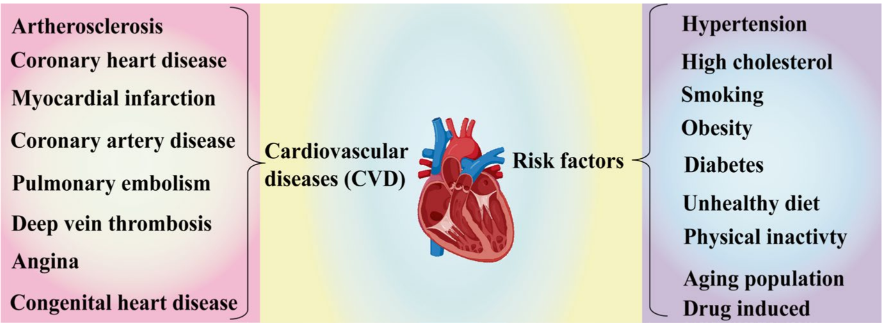
Artherosclerosis
Coronary heart disease
Myocardial infarction
Coronary artery disease
Pulmonary embolism
Deep vein thrombosis
Angina
Congenital heart disease
Cardiovascular diseases (CVD)
Risk factors
Hypertension
High cholesterol
Smoking
Obesity
Diabetes
Unhealthy diet
Physical inactivty
Aging population
Drug induced

Fig. 1 Different types of CVDs and associated risk factors

artery disease (CAD), and arterial hypertension (AH) are the main causes of CVDs. Among these atherosclerosis is the primary cause of cardiovascular-related deaths globally. It involves lipid accumulation inside artery walls, leading to various cardiovascular problems and other diseases [7]. High levels of cholesterol in the blood are a major factor in the development of atherosclerosis. CAD is a common heart condition characterized by the narrowing or blockage of major blood vessels known as coronary arteries [8]. Plaque formation within the artery wall is the primary cause of CAD, along with chronic inflammation. This restricts blood flow to the heart muscle, resulting in insufficient oxygen and nutrients supply [9]. If the atherosclerotic plaque ruptures, it can lead to thrombosis and complete vessel closure, causing heart attacks, strokes, limb ischemia, and death. Other factors contributing to CAD include diseased endothelium, low-grade inflammation, and lipid accumulation [7]. AH is one of the most prevalent CVDs and often shows no symptoms. However, it significantly increases the risk of heart attacks, strokes, kidney failure, and peripheral vascular disease [10]. AH is diagnosed when a person's blood pressure readings consistently show values above certain thresholds. In this chapter, we will provide an overview of the nanoparticle-based imaging modalities and therapeutic interventions in the context of cardiovascular diseases (CVDs).

2 Current Challenges in Cardiovascular Diagnosis and Therapy

One of the current challenges in cardiovascular diagnosis is the limited access to specialized testing, particularly for underserved populations [11]. Additionally, diagnostic errors are common in cardiovascular care, with missed or delayed diagnoses contributing to adverse outcomes for patients [12]. Furthermore, there is underutilization of non-invasive diagnostic tools, such as stress tests and echocardiograms, in the evaluation of patients with suspected cardiovascular diseases [13]. These challenges are compounded by cost and insurance barriers that can prevent patients from accessing necessary cardiovascular diagnostic tests, leading to delays in diagnosis and treatment [14]. Effective communication and coordination among healthcare providers involved in cardiovascular care are essential to ensure timely diagnosis and appropriate management of patients [15]. Addressing these challenges requires ongoing education and training for healthcare providers. Additionally, racial, and socioeconomic disparities exist in access to cardiovascular diagnostic services, with minority and low-income populations facing barriers to timely diagnosis and treatment [11]. Many systemic issues affect both patients and clinicians, such as the lack of communication between working staff, both clinician and doctors. There are several obstacles resulting from the existing protocol involving the selection procedure of patients to be selected by specialists for treatment. Several resource-related challenges prevent patients from receiving accurate and efficient diagnoses, including limited access to clinicians, investigations, and time. One of the current challenges in cardiovascular disease therapy is the underutilization of guideline-directed medical therapy (GDMT) in clinical practice. Despite evidence supporting the use of specific medications and interventions to improve outcomes in patients with cardiovascular diseases, studies have shown that GDMT is often not prescribed or optimized according to guidelines. Research highlights significant gaps in adherence to GDMT for heart failure patients, resulting in suboptimal management and a heightened risk of adverse events [16]. Another challenge in cardiovascular disease therapy is medication non-adherence among patients. Studies have demonstrated that a significant proportion of patients do not adhere to prescribed medications for cardiovascular conditions, which can lead to poor treatment outcomes and increased healthcare costs. Studies have reported the importance of medication non-adherence through patient education, counselling, and monitoring to improve medication adherence and outcomes in cardiovascular disease patients [17]. Furthermore, the rising costs of cardiovascular medications and interventions pose a significant challenge in ensuring access to effective therapies for all patients. Several studies highlight the financial burden of cardiovascular medications on patients, especially those with limited financial resources, leading to differences in access to essential treatments and suboptimal outcomes [18]. Addressing these challenges requires a multidisciplinary approach involving healthcare providers, policymakers, and patients to improve the delivery and affordability of cardiovascular disease therapies. Strategies such as implementing value-based care models, enhancing patient education and support programs, and advocating for policies that promote equitable access to cardiovascular therapies are essential to overcome these challenges and improve outcomes for patients with cardiovascular diseases.

3 Nanotechnology in the Management of CVDs

Nanoparticle-based systems are extensively being employed in the field of healthcare due to ability to augment the safety, stability, and bioavailability of already existing medications. The field of cardiovascular nanomedicine primarily focuses on developing novel approaches to address the limitations of CVD diagnosis and treatment. Nanomaterials possess unique physicochemical properties like ultra small size, shape, biocompatibility, interconnected macropores, high surface area to volume ratio, high surface energy, tuneable porosity and facile surface modification/functionalization enabling them to impact cellular processes without causing harm to normal cells [19, 20]. A wide range of nanoparticles like polymeric nanoparticles, polymeric micelles, lipid nanoparticles, metal-based nanoparticle, silica nanoparticles, nanofibers, nanocrystals and quantum dots are being utilised for the targeted and accurate delivery of medications that regulate lipid abnormalities, reduce inflammation and angiogenesis in atherosclerotic plaques, and prevent plaque thrombosis (Fig. 2) [21]. These nanoparticles are known for encapsulating a wide range of therapeutic agents to combat CVDs. In the realm of nanomedicine, encapsulation serves dual functions. Firstly, it acts as a protective shield in vivo, safeguarding the body from potential toxicity of drugs and ensuring controlled release through the intrinsic properties of the encapsulating material. Additionally, surface modifications enable targeted delivery to the affected site. Further, the encapsulation process protects the therapeutic agents from undesired modifications and degradation, thereby enhancing their stability and efficacy [22]. Nanoparticles (NPs) exploit the higher permeability and retention effect to accurately transport medications to affected sites, leading to improved therapeutic outcomes and reduced tissue

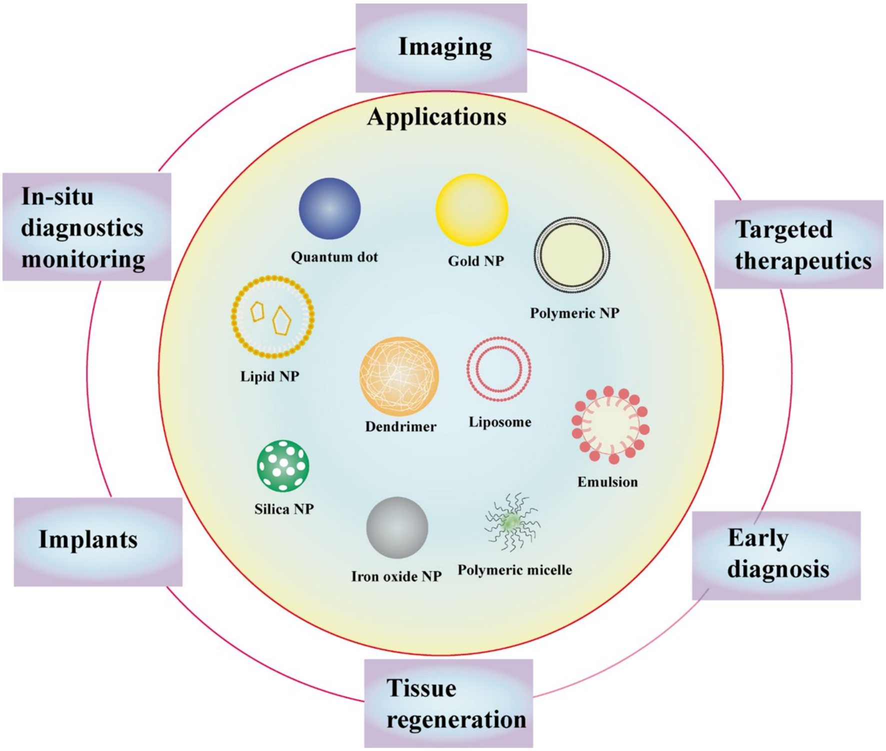
Imaging
Applications
In-situ diagnostics monitoring
Targeted therapeutics
Quantum dot
Gold NP
Polymeric NP
Lipid NP
Dendrimer
Liposome
Emulsion
Silica NP
Iron oxide NP
Polymeric micelle
Implants
Early diagnosis
Tissue regeneration

Fig. 2 Different types of NPs and their applications in CVDs

harm [23, 24]. Recently, the ability of nanocarriers in leveraging the inherent properties of circulating cells emerged as a prominent area of interest. In order to evade the macrophage phagocytosis and mitigate the immunogenicity, NPs are integrated with cell carriers like red blood cells or platelets showing superior therapeutic efficacy compared to conventional drug delivery mechanisms [19] . Furthermore, nanoparticle-based drug delivery systems exhibit significant promise in enhancing therapeutic effectiveness, extending drug duration, enhancing drug absorption, passively or actively targeting specific areas, mitigating drug resistance, and minimising adverse drug responses [25].

4 Nanotechnology-Based Imaging Techniques for Cardiovascular Diseases

In recent years, nanotechnology has offered new materials and strategies for more effective diagnosis of CVDs. Multiple biomarkers have been investigated and utilised for the detection of CVDs. However, traditional approaches also need costly and time-consuming blood test analyses. Cardiac biomarkers present in human bodily fluids are reliable and accurate indicators of the risk and progression of CVDs [26]. The levels of cholesterol and lipoproteins like very low-density lipoproteins (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) are well-recognised as reliable predictors of an unhealthy cardiovascular state [27]. Cardiac biomarkers present in human bodily fluids, such as C-reactive protein (CRP), D-dimer, MCP-1, cardiac troponins I and T (cTnI and cTnT), B-type natriuretic peptides (BNP and NT-proBNP), and TNF-α, are reliable and accurate indicators of the risk and progression of CVDs [26, 28, 29]. Some of the most commonly used biomarkers of CVDs are highlighted in Table 1.

Various imaging techniques such as Electrocardiogram (ECG), ultrasound imaging, coronary angiography (CA), Magnetic Resonance Imaging (MRI), Computed Tomography (CT), positron emission tomography (PET), and single-photon emission CT (SPECT) are currently used for visualizing CVDs [34]. Despite significant advancements in CVD imaging, these methods often require large amounts of contrast agents, and misinterpretation of results can lead to incorrect diagnoses [35–37]. There is a critical need for more efficient diagnostic tools and safe, non-invasive imaging techniques to obtain precise information on CVDs. Nanotechnology involves materials with dimensions at the nanoscale, possessing unique optical and electrical properties and excellent biocompatibility, making them highly attractive for enhancing current diagnostic procedures. One early application of nanotechnology in CVDs involves its use as a contrast agent in MRI. For instance, dextran-coated ultra-superparamagnetic iron oxide nanoparticles have been employed with MRI to visualize rejection sites following cardiac allograft transplantation in rats. These nanoparticles bind to macrophages, aiding in the early detection of inflammatory reactions [38]. In another study, researchers developed VINP-28, a novel

Table 1 Common Biomarkers of CVDs

Biomarkers	Condition	References
CRP	Cardiovascular Morbidities, Artherosclerosis	[26]
BNP and NT-proBNP	Heart Failure	
D-dimer	Ischemic heart disease, thrombosis acute aortic dissection and cardiovascular death	
cTnI and cTnT	Acute Myocardial Infarction	
Glycogen phosphorylase BB (GPBB)	Acute Coronary Syndrome (ACS), Myocardial Ischemia, Acute Myocardial Infarction	[30]
Aspartate aminotransferase (AST)	Acute myocardial infarction (AMI)	[31]
Lactate dehydrogenase (LDH)	Differentiate acute from subacute myocardial infarction (MI)	[32]
Mid-regional pro-a-type natriuretic peptide (MR-pro-ANP)	Acute Heart Failure (HF)	[33]

targeted agent consisting of monocrystalline magnetic nanoparticles (MNPs), which can detect initial signs of inflammation in atherosclerosis in murine models. VINP-28 targets vascular cell adhesion molecule-1 (VCAM-1) and shows co-localization with endothelial cells and other VCAM-1-expressing cells, such as macrophages. Moreover, it exhibits a distinct spatial distribution compared to untargeted control nanoparticles. In mice treated with atorvastatin, a decrease in VINP-28 deposition and VCAM-1 expression correlated with the presence of atheromata, suggesting potential clinical utility for identifying early inflammation in atherosclerosis without invasive procedures [39]. In a study by McCarthy et al., magneto-fluorescent nanoparticles (MFNP) coated with dextran were developed with a photosensitizer attached to it, that specifically targeted plaque macrophages. The primary amines in MFNP nanoparticles were chemically bonded to 5-(4-carboxyphenyl)-10,15,20-triphenyl-2,3-dihydroxychlorin (TPC), a highly effective photosensitizer. This enabled the nanoparticles to generate singlet oxygen when exposed to light of a specific wavelength, demonstrating potential for identifying and eliminating macrophages at atherosclerotic plaque sites [40].

5 Nanoparticles for Targeted Drug Delivery in Cardiovascular Therapy

Nanotechnology-based targeting strategies in cardiovascular therapy can be broadly classified into two categories: passive targeting and active targeting. Passive targeting relies on the physicochemical characteristics of cells and the surrounding environment. In contrast, active targeting utilizes cell-specific ligands to achieve precise and highly effective binding to surface receptors or transporters on heart cells or other cells within the heart [41]. The passage of nanocarriers loaded with drugs through vascular walls is challenging due to the strong interconnection of microvascular endothelial cells in healthy tissues and the high molecular weights of these carriers. However, during the progression of atherosclerosis, heightened vascular permeability and inflammation, triggered by the release of cytokines, create pathways for nano-drugs to traverse from the inner surface of blood vessels into plaques. This phenomenon is known as the enhanced permeability and retention (EPR) effect, facilitating the passive entry of nanodrugs into affected myocardial tissues [42]. It is important to note that the EPR effect is transient as vascular permeability returns to normal within a few days [43]. Another passive targeting approach involves surface modification of NPs to allow them to covalently bind to circulating cells, such as inflammatory cells (monocytes or macrophages). These modified NPs can migrate to the heart, taking advantage of the high permeability and retention of diseased blood vessels [44]. Thus, passive targeting mainly relies on high permeability of leaky vasculature and retention of diseased blood vessels. In addition to passive targeting, active targeting strategies aim to enhance the efficiency of drug delivery to CVDs and involves modifying nanocarriers by incorporating specific targeting agents or creating stimulus-responsive smart nanocarriers (Fig. 3). This modification enables drugs to precisely reach their intended locations, offering superior intra-lesion localization and potentially fewer side effects over a shorter duration compared to passive targeting [45].

The size, shape, and surface features of nanomaterials significantly affect their biodistribution, pharmacokinetics, and half-life, as well as on the targeting efficiency [46]. Smaller nanomaterials are readily eliminated by the kidneys, whereas larger ones are more readily taken up by the reticuloendothelial system in the liver and spleen [47]. The morphology of nanomaterials also influences their interaction with cells, duration of circulation in the body, and tendency to accumulate at the periphery of blood vessels. Nonspherical nanoparticles are preferred for targeting atherosclerosis due to their enhanced circulation in the bloodstream and increased cellular uptake. Different shapes also show a preference for various subpopulations of inflammatory cells [48]. Surface characteristics, such as surface charge, impact cellular absorption and blood circulation duration. Nanomaterials with a positive charge are more easily taken up by cells, whereas those with a negative charge are hydrophobic and can be quickly removed by macrophages. Surface modification can effectively tackle these problems by applying hydrophilic polymers as a coating on nanomaterials, thus creating a hydrated outer layer [49]. These coatings can transform functional components into nanomaterials, altering their targeted performance, biodistribution, immunogenicity, and toxicity, while also providing them with new capabilities.

6 Nanomedicine Approaches for Treatment of CVDs

Liposomes are lipid-based nanoparticles with an amphiphilic structure, enabling them to effectively transport and administer medications with diverse characteristics such as hydrophilicity, hydrophobicity, and lipophilicity. Additionally, liposomes may simultaneously absorb both hydrophilic and lipophilic substances.

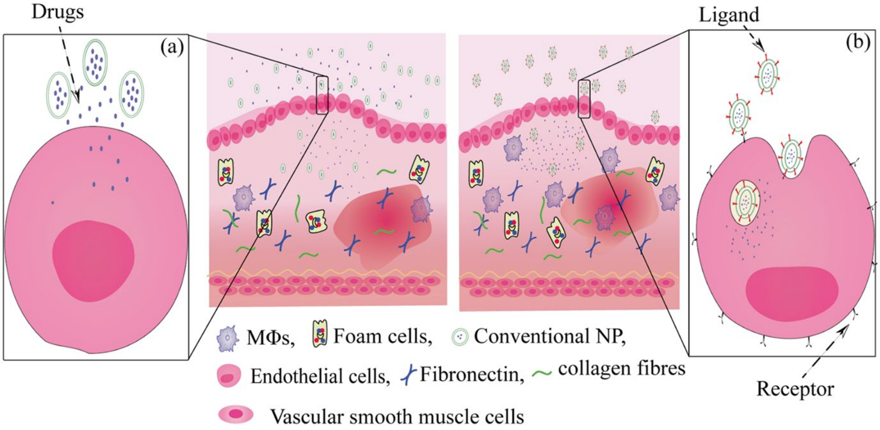

Fig. 3 Targeting Strategy of NPs in CVD (**a**) Passive Targeting is achieved through the passive diffusion of NPs containing drugs through the endothelial cells (Non-specific uptake) (**b**) Active targeting involves targeting ligands on the surface of nanoparticles (NPs) to bind with receptors on endothelial cells leading to selective uptake of the NPs by receptor-mediated endocytosis

Lobatto et al. developed a nanoformulation using liposomes for the prevention and treatment of atherosclerosis. This formulation utilises glucocorticoids to reduce the number of macrophages in the plaque region by taking advantage of blood vessel permeability. It has been found to have antiangiogenic effects and does not cause any toxicity in rabbit models [50]. Another group developed a nanofomulation of Amiodarone, a medication used to treat irregular heart rhythms but has severe effects on blood circulation. The liposomal-amiodarone was introduced into the ischemic/reperfused myocardium, resulting in decreased mortality from deadly arrhythmia and unfavourable hemodynamic consequences in rat models [51]. Same researchers showed that adenosine enclosed within liposomes coated with polyethylene glycol (PEG) exhibited improved cardioprotective effects and reduced adverse effects in a rat model of ischemia/reperfusion. PEGylated liposomal adenosine exhibited an extended duration in the bloodstream and resulted in a smaller myocardial infarction (MI) size as compared to saline [52]. Another investigation examined the effectiveness of PEGylated Liposomes in treating Myocardial Infarction in rat and mouse models. The study discovered that nanosized PEGylated liposomes were capable of transporting growth factors and cytokines to the damaged heart tissue [53]. Another study showed that the introduction of recombinant high-density lipoprotein (rHDL) containing Pitavastatin resulted in improved targeting of macrophages in plaques and a reduction in plaque area in both cell cultures and mice models of atherosclerosis [54].

Polymers are extensively being utilized as drug carriers due to their reduced toxicity in comparison to metal carriers and presence of chemical groups for attachment with the target. Biodegradable polymers like polylactic acid (PLA), polyglycolic acid, poly lactic-co-glycolic acid (PLGA) and poly(ε-caprolactone) (PCL) have been used in various drug delivery applications [55]. In one of the first studies conducted to assess the efficacy of nano-polymeric drug delivery systems in treating restenosis using an *ex-vivo* vascular model, the (PLGA) nanoparticles were synthesised and subsequently subjected to surface modification using a cationic agent leading to ten times greater uptake in arteries [56]. Polymeric nanoparticles are frequently either nanocapsules or nanospheres. In nanocapsules, the therapeutic component is enclosed within a polymeric capsule shell, while in nanospheres, drugs or other solid particles are embedded in a polymeric matrix [57]. PGLA Nanoparticles encapsulating pitavastatin inhibited monocyte migration induced by monocyte chemoattractant protein-I (MCP-1) and suppressed the secretion of MCP-1 and matrix metalloproteinase-9 from cultured macrophages, thereby preventing destabilisation of atherosclerotic plaques and subsequent acute myocardial infarction in mouse models [58]. Prostacyclin analogue, beraprost (BPS) incorporated into PGLA nanoparticles injected into the trachea of monocrotaline rat models of pulmonary arterial hypertension exhibited a reduction in pulmonary vascular resistance and suppression of pulmonary vascular remodelling [59]. Nanoparticles composed of PGLA containing irbesartan suppressed the employment of inflammatory monocytes, decreased the amount of tissue damage caused by lack of blood flow and subsequent restoration, and improved the restructuring of the left ventricle in mice models of AMI with myocardial IR injury [60]. PLGA-stearylamine nanoparticles, which include heparin and glutathione, demonstrated remarkable cellular absorption specifically at the site of ischemia/reperfusion (I/R) injury in human bone marrow mesenchymal stem cells (hBMSCs). These nanoparticles possess both anticoagulant and antioxidant characteristics. This approach has the potential to be employed in the management of several vascular disorders [61]. In a separate investigation, PGLA nanoparticles containing pioglitazone were used to control inflammation in the cardiovascular system of a mouse model with atherosclerosis. This was achieved by regulating the levels of both inflammatory and non-inflammatory cytokines in the peripheral blood and stabilising plaques. As a result, the nanoparticles were able to prevent life-threatening conditions such as acute myocardial infarction (AMI) [62].

Dendrimers are intricate polymers that possess precise physicochemical characteristics. They are utilised for drug delivery because of their moderate surface areas, high molecular masses, low viscosities, and excellent biocompatibility. Polyamide amine dendrimers can inhibit the platelet aggregation, generating anti-thrombotic effects and repairing endothelial injury [63]. PEGylation The PAMAM dendrimer, modified with arginine-glycine-aspartic peptides (RGDyC) and the recombinant hirudin gene (rHV), showed antithrombotic and anticoagulant properties in the venous thrombosis model of Wistar rats, suggesting potential for thrombus targeting therapy [64] Gold, silver, and iron are the most frequently utilised metal nanoparticles as nanocarriers due to their reduced size and unique physical, chemical, and biological characteristics. A study investigating spherical gold nanoparticles (AuNPs) containing Levosimendan (Simdax) in heart cells of rats with doxorubicin-induced heart failure found improved protection of heart, when delivered using sonoporation [65]. Another intriguing research found that combining Copper Sulphide (CuS) nanoparticles with antibodies targeting TRPV1 cation channels decreased the lipids build up in mice models lacking the ApoE gene, demonstrating the effectiveness in combating the development of atherosclerosis [66]. Iron Oxide NP combined with tissue plasminogen activator (tPA), specifically targeted tPA in the stenotic arteries of swine models with thrombosis, increasing concentration of the drug at the lesion site. Therefore, this approach can be utilised for various vascular conditions, such as treating atherosclerotic plaques and ischemic stroke [67]. Micelles, another type of nanoparticles formed by self-assembling lipids or amphiphilic molecules, are smaller and more compact. They serve as compartments for insoluble pharmaceuticals in drug delivery systems, targeting specific plaque components. Micelles' higher permeability allows them to target lesions in the ischemic myocardium [68] In a recent study involving ApoE−/− mice, peptide micelles containing microRNA-145 were shown to enter vascular smooth muscle cells (VSMCs) more effectively. The micelles prevented the development of early and late stages of atherosclerosis by suppressing cell types contributing to plaque formation and restoring markers associated with protection against atherosclerosis [69].

7 Concluding Remarks

Nanomaterials have significantly transformed the diagnosis and treatment of cardiovascular diseases (CVDs) by augmenting the effectiveness of conventional materials. The remarkable potential of nanoparticles in revolutionizing CVD management is evident across various fronts. From targeting atherosclerosis, myocardial infarction, stroke, pulmonary hypertension, to cardiomyopathy, nanoparticles offer a multifaceted approach to treatment. Although nanotechnology has advanced drug delivery systems in disease treatments, it has several limitations, including a lack of clinical trial and safety data, tedious methods for characterization and purity analysis, high costs of scale-up production, and concerns about the long-term stability of drugs [70]. This hurdle can be overcome by collaboration among bioengineers, pharmacists, chemists, biologists, and clinicians. Future studies aim to utilize peptides and antibodies for identifying cardiovascular disease markers and creating targeted nano delivery systems [71]. In essence, the convergence of nanotechnology and cardiovascular medicine represents a paradigm shift in disease management, promising enhanced efficacy, reduced side effects, and improved patient outcomes. As research in this field continues to evolve, nanoparticle-based interventions hold the potential to revolutionize the landscape of cardiovascular care, offering new avenues for diagnosis, treatment, and prevention.

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Employing Nano-Extracellular Vesicles (NEVs) as a Potent Therapy in Ameliorating Bone Loss in Osteoporosis

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1 Introduction

Osteoporosis is a metabolic skeletal disease that is characterized by demineralization of bone, low bone mass and increased fracture risk [1, 2]. Osteoporosis is also addressed as a ‘silent disease of bones’ due to its asymptomatic nature until fractures occur, which can result from minor trauma, falls, or even routine activities like coughing or sneezing [3, 4]. These fractures lead to persistent pain, physical disabilities and increased mortality rates, particularly evident with hip fractures where 20–24% of patients succumb within a year [5–9]. According to the National Osteoporosis Foundation, 1 in 2 women and 1 in 4 men over the age of 50 suffer from fractures attributable to osteoporosis. It is estimated that in Asian countries the number of hip fractures will increase up to 2.56 million in 2050 from 1.12 million in 2018 increasing the economic burden of treatment costs from 9.5 billion USD to 15 billion USD [10]. With changing lifestyles and an aging population, osteoporosis incidence rates are surging worldwide, posing substantial socio-economic burdens. All the available osteoporosis treatments primarily aim to reduce fracture risk by targeting bone resorption. For patients at higher fracture risk, a combination of non-pharmacological and pharmacological approaches is employed. Pharmacological treatments are categorized by their mode of action and include anti-resorptive drugs (estrogen, bisphosphonates, selective estrogen receptor modulators, calcitonin, denosumab), osteo-anabolic drugs (teriparatide, abaloparatide), and dual-acting drugs (romosozumab) [11, 12]. Despite their efficacy, osteoporosis medications may induce adverse effects such as atypical femur fractures, osteonecrosis of the

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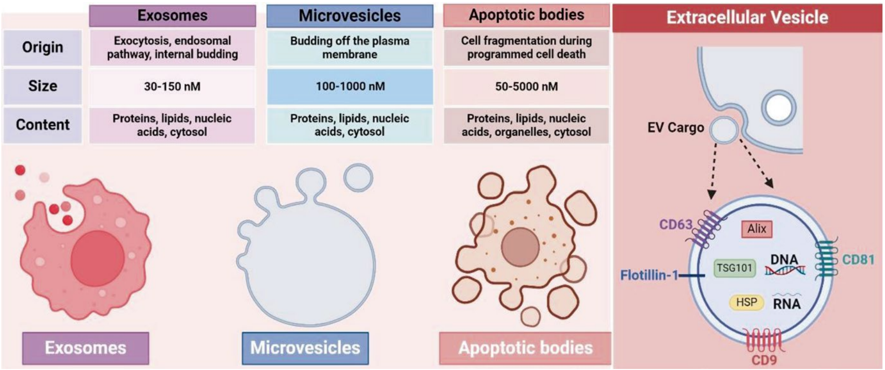
jaw, and bone/joint pain [13, 14]. Thus, there is a need for precision medications with fewer side effects.

Extracellular Vesicles (EVs) are membrane-bound vesicles released from almost every cell and serve as a crucial mediator of cell-cell communication [15]. EVs facilitate the exchange of information between cells via the transportation of molecular cargo such as proteins, messenger ribonucleic acid (mRNA), microRNA (miRNA), and deoxyribonucleic acid (DNA) [16, 17, 33]. Importantly, the composition of EV cargo reflects the physiological and phenotypic characteristics of the donor cell [18, 19]. Investigations into the molecular cargo of EVs have unveiled their potential utility as diagnostic and prognostic biomarkers for several diseases. Furthermore, research has increasingly explored the therapeutic applications of EVs in various medical conditions, including osteoporosis [19–21]. EVs have emerged as a promising treatment option for osteoporosis, showing efficacy in both *in vitro* and *in vivo* studies [22–26]. Notably, EVs offer the advantages of minimal toxicity and immunogenicity, making them a safe and efficient therapeutic approach [27]. In the context of osteoporosis, EVs derived from diverse sources such as mesenchymal stem cells (MSCs), bone cells (osteoclasts, osteoblasts, and osteocytes), immune cells, microbes, and plant origins have emerged as promising candidates for disease management and treatment. Both native and engineered EVs have been investigated in preclinical studies and have demonstrated encouraging results in mitigating osteoporotic manifestations [25, 28–31]. This book chapter offers a comprehensive review of recent research focusing on the theragnostic potential of EVs, underscoring their role in the management and treatment of osteoporosis.

2 Extracellular Vesicles (EVs)

EVs are a broad term used to represent lipid membrane-bound, non-replicating nanoscale vesicles released from almost all types of cells (MISEV 2023) [32]. EVs are recognized to play a crucial role in intercellular communication via the translocation of various cargo molecules, including proteins, amino acids, lipids, and nucleic acids (DNA, mRNA, and miRNA) from the parent cell to the recipient cell [15, 17, 33, 34]. EVs are enriched in specific proteins, such as cluster of differentiation (CD)-63, CD81, CD9, tumor susceptibility gene 101 protein (TSG101), ALG-2-interacting protein X (Alix), heat shock protein (HSP)-70, HSP-90, and flotillin-1, which play critical roles in biogenesis, recipient cell recognition, and uptake [35–37]. However, EVs unique composition varies greatly, containing different kinds of carbohydrates, nucleic acids, and proteins depending on the physiological state of the parent cell [38, 39]. Based on the biogenetic origin pathway, EVs are broadly classified into three main categories: Exosomes, Microvesicles (Ectosomes/Microparticles) and Apoptotic bodies [16, 40] (Fig. 1). These three types of EVs differ from one another in terms of size, biological function, molecular cargo, and biogenesis and release mechanism. Exosomes are EVs that are formed in the endosomal compartment of cells and range in size from 30 to 150 nm. Microvesicles (MVs) are EVs that are released from the cell membrane and have a size range of



	Exosomes	Microvesicles	Apoptotic bodies
Origin	Exocytosis, endosomal pathway, internal budding	Budding off the plasma membrane	Cell fragmentation during programmed cell death
Size	30-150 nM	100-1000 nM	50-5000 nM
Content	Proteins, lipids, nucleic acids, cytosol	Proteins, lipids, nucleic acids, cytosol	Proteins, lipids, nucleic acids, organelles, cytosol

Fig. 1 Types of Extracellular Vesicles (EVs). Based on the biogenetic origin pathway, EVs are broadly classified into three main categories: Exosomes, Microvesicles and Apoptotic bodies. EVs are enriched in specific proteins, such as a cluster of differentiation (CD)-63, CD81, CD9, tumor susceptibility gene 101 protein (TSG101), ALG-2-interacting protein X (Alix), heat shock protein (HSP), and flotillin-1, which play critical roles in biogenesis, recipient cell recognition, and uptake

100–1000 nm. Apoptotic bodies are EVs produced from cells undergoing programmed cell death and range between 50 and 5000 nm in diameter [41]. Although EVs are categorized into three main types, distinguishing the specific type present in isolated fractions remains challenging due to the heterogeneous nature of most samples. This complexity makes it difficult to differentiate EVs solely based on size [42]. To address this issue and provide clarity regarding EV classification, György et al. proposed the term “extracellular vesicles” to encompass all types of vesicles released from cells [41]. This terminology has been adopted in the Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines, endorsed by the International Society for Extracellular Vesicles (ISEV), to refer to all vesicle types present in isolated fractions [42]. This standardized terminology facilitates communication and interpretation of research findings in the field of EV biology.

Beyond their role in intercellular communication, EVs serve as diagnostic and prognostic biomarkers for diseases and hold promise as therapeutic tools due to their ability to cross biological barriers, minimal cytotoxicity and immunogenicity, target specificity, and efficient cargo delivery [43–45]. Despite their potential, challenges such as low purity, recovery, and limited targeting ability hinder their therapeutic application [46]. Strategies like EV engineering aim to enhance their therapeutic effects by improving cargo delivery and targeting specificity [47].

3 Isolation and Characterization of EVs

The amount of research in this field of EVs has been growing swiftly, leading to the development of numerous techniques for isolating and characterizing them. To isolate EVs, a variety of biological materials can be utilized, including blood, urine, saliva, tissues, cerebrospinal fluid (CSF), amniotic fluids, milk, bacteria, plant cells, cell lines etc. [48, 49]. Different isolation techniques exist, each influenced by the physical and biological properties of EVs, such as size and surface molecules, with variations in purity and yield [50]. For EVs to be used as a treatment and to prevent adverse effects during clinical administration, very pure EV isolation free of contaminating proteins, lipoproteins, and nucleic acids is clinically crucial [51].

The gold standard for EV isolation is ultracentrifugation (UC), which separates EVs according to their size and density while using a high centrifugation speed [52, 53]. Two forms of UC, differential centrifugation and density gradient centrifugation are commonly used in EV isolation. Precipitation is another common EV isolation technique that is based on the physiochemical characteristics of EVs. Precipitation methods alter EV solubility using buffers such as polyethylene glycol, sodium acetate, acetone, and hydrophilic polymers to precipitate them at low centrifugation speeds [50, 54]. Commercial kits based on precipitation principles are also available [52, 55]. Additionally, techniques such as ultrafiltration, size exclusion chromatography, immunoaffinity capture, and microfluidics are employed for EV isolation, each has advantages and disadvantages related to sample volume, sample purity, recovery, isolation time, and handling [52, 53]. Standardization of an appropriate isolation technique that produces repeatable and trustworthy findings is necessary to enable the clinical applications of EVs.

Following isolation, EVs must undergo characterization to confirm their presence, purity, concentration, and molecular composition. Various methods are recommended by the MISEV guidelines, including dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), tunable resistive pulse sensing (TRPS), and imaging techniques such as transmission electron microscopy (TEM), Cryo-EM, Immunogold-EM, and Scanning EM and atomic force microscopy (AFM) [49]. Western blotting and flow cytometry techniques are also employed for EV characterization that rely on the presence of certain markers on EVs. Western blotting is commonly used to evaluate EV marker proteins and purity, with recommended markers including CD63, CD81, CD9, TSG101, Alix, and HSC70 [49]. It is also important to assess the presence of co-isolated contaminants, such as albumin (serum/plasma), apolipoproteins (serum/plasma), calnexin, and Tamm-Horsfall protein (urine) to evaluate purity [42]. Accurate EV characterization is pivotal for ensuring the reliability and reproducibility of research outcomes and interpreting data for potential therapeutic applications. Consequently, the establishment of standardized protocols for both EV isolation and characterization is imperative to drive forward the clinical utility of EVs effectively.

4 EVs as a Theragnostic Tool of Osteoporosis

EVs convey their molecular cargo—DNA, miRNA, mRNA, and proteins—between cells to facilitate communication. The composition of EV cargo reflects the physiological and phenotypic state of the parent cell, making it a potential diagnostic biomarker for various disorders. Moreover, emerging research indicates that the molecular cargo encapsulated within EVs holds therapeutic potential for the treatment of certain illnesses. In the following sections, we provide a comprehensive summary of both *in vitro* and pre-clinical studies about the diagnosis and therapy of osteoporosis, elucidating the potential applications of EV-based approaches in addressing this prevalent skeletal disorder.

4.1 EVs in Diagnosis of Osteoporosis

Efforts to elucidate a diagnostic biomarker for osteoporosis have led to investigations into the molecular cargo present in circulating EVs of affected individuals. Vesicular non-coding RNAs (ncRNAs) (miRNA, CircRNA, lncRNA, etc.) & proteins have been evaluated as potential biomarkers for osteoporosis [56]. This comprehensive approach aims to identify reliable molecular signatures that could aid in the early detection and monitoring of osteoporosis, offering insights into its pathophysiology and progression. Serum exosomes from postmenopausal women with and without osteoporosis differed in the expression of miRNAs related to the Wnt, mitogen-activated protein kinases (MAPK), and hippocampal signaling pathways suggesting that miRNAs may play a role in elevating the risk of osteoporosis and may be used as a potential biomarker [57]. Sun et al. investigated the levels of miR-214 in the serum exosomes of patients with osteoporosis and healthy controls. They observed that the expression of miR-214 was significantly upregulated in the exosomes of patients with osteoporosis, indicating the possibility of using miR-214 as a diagnostic biomarker for osteoporosis. Additionally, they revealed that miR-214 in exosomes has a role in preventing osteoblastogenesis [58]. A different study evaluated the differential miRNA expression profile in the plasma exosomes of postmenopausal women who were classified according to their bone mineral density (BMD) as healthy controls, osteopenic, or osteoporotic. The expression of hsa-miR-642a-3p was shown to differ across the three groups, indicating that the presence of this biomarker in the osteopenic group acts as a prognostic indicator for osteoporosis [59]. Another study indicated that osteoporotic women had higher levels of receptor activator of nuclear factor kappa beta ligand (RANK-L)$^{+}$ EVs in the plasma. When compared to controls, it was observed that exosomal miR-1246 was upregulated in osteoporotic and osteopenic individuals. Additionally, individuals with Gorham-Stout disease also had increased levels of miR-1246, which may indicate that miR-1246 functions as a biomarker for bone loss diseases [60]. Shi et al. compared the miRNA expression profile in the serum exosomes of postmenopausal women with osteoporosis-related fractures and postmenopausal women with normal bone mass. Potential diagnostic biomarkers of osteoporosis were identified to be mir-324-3p, mir-330-5p, mir-766-3p, mir-1247-5p, and mir-3124-5p [61].

Zhang et al. (2018) examined the expression levels of transfer RNA-derived fragments (tRFs) in the plasma exosomes of healthy controls and osteoporotic patients and identified exosomal tRF-18, tRF-25 and tRF-38 as a potential diagnostic biomarker for osteoporosis [62]. Analysis of the lncRNA profile in serum exosomes from osteoporotic and non-osteoporotic patients identified 296 lncRNAs were up-regulated and 96 were down-regulated in osteoporotic individuals and this differential lncRNA profile can be further explored for developing diagnostic biomarkers for osteoporosis [63]. Additionally, it was discovered that the expression of circular RNAs varied between osteoporotic patients and healthy controls. Hsa_circ_0006859 was shown to be increased in serum exosomes of osteoporotic patients using CircRNA microarray, suggesting Hsa_circ_0006859 as a biomarker for osteoporosis [64].

In addition to the ncRNA biomarker profile, studies have also identified proteins that could serve as diagnostic biomarkers for osteoporosis. Evaluation of the proteomic profile of human serum MVs from normal, osteopenic, and osteoporotic individuals identified nearly 200 differentially expressed proteins in the serum MVs and 19 proteins were upregulated and 5 were downregulated in both osteopenic and osteoporotic groups. Profilin-1 was found to be significantly upregulated in osteoporotic patients suggesting its potential as a diagnostic biomarker for osteoporosis [65] (Table 1). In another study, forty-five proteins were found to express differently in the plasma exosomes of patients with normal, osteopenic, and osteoporotic BMD. Out of 45, four proteins AARS, PCPB2, PSMB9, and VSIR were further validated to be associated with osteoporosis [66]. These findings demonstrate the potential utility of proteomic analysis in identifying biomarkers for osteoporosis diagnosis. Altogether, these studies highlight the need for additional research to identify an efficient diagnostic biomarker that could aid in the prediction and early diagnosis of osteoporosis.

Table 1 EVs associated diagnostic biomarkers for osteoporosis

EVs source	Diagnostic biomarker	Expression	References
Serum	miR-214	Upregulated in osteoporotic patients	[57]
Plasma	hsa-miR-642a-3p	Upregulated in post-menopausal osteoporotic women	[58]
Plasma	miR-1246	Upregulated in post-menopausal osteoporotic women	[59]
Serum	miR-324-3p, miR-330-5p, miR-766-3p, miR-1247-5p, miR-3124-5p	Upregulated in post-menopausal osteoporotic women	[60]
Plasma	tRF-18, tRF-25, tRF-38	Upregulated in osteoporotic patients	[61]
Serum	Has_circ_0006859	Upregulated in osteoporotic patients	[63]
Serum	Profilin-1	Upregulated in osteoporotic patients	[64]

4.2 EVs in the Therapy of Osteoporosis

For therapeutic purposes, EVs have been isolated from diverse sources, including stem cells, bone cells, bone-associated cells, bacteria, and plants. In this section, we provide an extensive and current assessment of these EVs in this area to facilitate the development of efficient therapeutic strategies in the management of osteoporosis. Although EVs derived from stem cells have been extensively reviewed in numerous publications, their therapeutic effects in osteoporosis are well-documented and thus not within the scope of this study. Researchers interested in exploring the therapeutic potential of various stem cells in osteoporosis are recommended to consult existing review articles for comprehensive insights [67–70].

EVs from Bone Cells

Osteoblast

Osteoblasts are bone-forming cells differentiated from mesenchymal stem cells (MSCs) and are responsible for the formation and mineralization of new bone [71, 72]. These cells aid in the production of new bone at the site of bone damage or fracture. Through the production of RANK-L and OPG, which are the regulators of osteoclast cell activity, osteoblast cells regulate the bone remodelling cycle [73]. It is observed that exosomes derived from osteoblasts have bone forming ability. In a study, EVs isolated from cell culture medium of early, middle and middle to late stages of osteoblastogenesis-induced MC3T3 cells were evaluated for their osteoblastogenic potential (Fig. 2 and Table 2). It was observed that EVs from mid to late-stage cells stimulated osteoblastogenesis *in vitro* in primary bone marrow mesenchymal stem cells (BMMSCs) and also enhanced bone formation *in vivo* in ovariectomized (ovx) mice model of Kunming mice suggesting the potential of osteoblast derived EVs in treating osteoporosis [22]. It is reported that exosomes isolated from mineralizing MC3T3 osteoblast cells (MOBs) increased Wnt/β-catenin signaling and altered the miRNA profile of ST2 cells, which in turn promoted osteoblastogenesis in ST2 cells [74]. It is observed that miR-503-3p overexpressing MC3T3 exosomes inhibited osteoclastogenesis via downregulating heparinase (Hpse) gene [75]. All these studies suggest the potential application of osteoblast EVs in therapies and imply the possible involvement of these cells in improving bone health.

Osteocytes

Osteocytes are the most abundant (almost 95% of bone cells) residing within the bone matrix [76]. Osteocytes are mechanosensory cells that regulate the activity of osteoblast and osteoclast cells, which in turn regulate the bone remodeling cycle [77]. EVs secreted from osteocytes communicate with both osteoclast and osteoblast cells to regulate their activity. Studies have reported that regulatory proteins

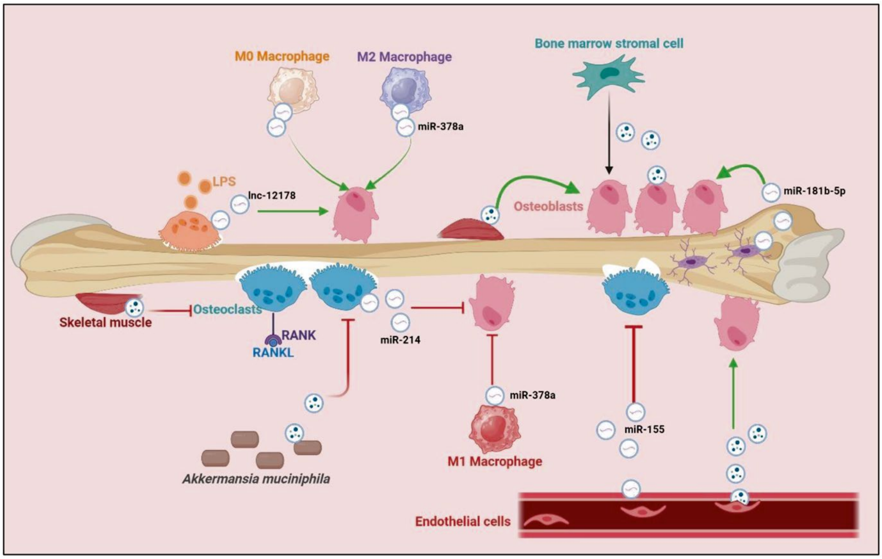


Fig. 2 Extracellular Vesicles (EVs) in the therapy of osteoporosis. EVs from the osteoblast cells promote osteoblastogenesis of primary bone marrow stromal cells. EVs from the osteocytes containing the miR-181b-5p also enhanced the osteogenic differentiation. Exosomes from RANK-L-induced osteoclasts contain miR-124 and inhibit osteoblastogenesis. EVs derived from LPS-induced osteoclasts on the other hand promote osteoblastogenesis. EVs from M0 and M2 cells improve bone healing but M1 EVs impede bone repair. Epithelial cell-derived exosomes promote osteoblastogenesis and inhibit osteoclastogenesis. EVs from the bacteria such as *Akkermansia muciniphila* also regulate bone health by inhibiting osteoclastogenesis

such as sclerostin (SOST) and RANK-L are more abundant in EVs that are released from osteocytes [78, 79]. It has been demonstrated that mechanically stimulated MLO-Y4 cells produced exosomes that were abundant in miR-181b-5p (Fig. 2 and Table 2). These EVs enhanced the osteogenic differentiation and proliferation of human periodontal ligament stem cells [80]. Mechanical stimulation of osteocytes regulates EVs release from osteocytes in Ca^{2++} dependent manner and has been shown to promote EVs production and increased protein loading illustrating the role of external forces in osteocyte vesicle release [78]. Exosomes from senescent MLO-Y4 cells inhibited osteogenesis of MC3T3-E1 cells and accelerated age-related bone loss in senescence-accelerated mouse P6 (SAMP6). It was observed that miR-494-3p was significantly downregulated in these exosomes, which led to activation of phosphate and tensin homolog (PTEN) which further suppresses the PI3K/AKT pathway to prevent osteoblastogenesis. Thus, this study suggests that the PTEN/PI3K/AKT pathway and miR-494-3p may be the targets for future osteoporosis treatments in the elderly [23]. Therefore, EVs derived from osteocytes can be effective in preventing bone loss however the potential of EVs generated from osteocyte cells in the treatment of osteoporosis can be better defined with more research on osteocyte EVs.

Table 2 EVs from bone cells in osteoporosis therapy

Sample/ source	Isolation method	Characterisation method	Cargo molecule	Model	Dosage	Effect	References
Cell culture supernatants of MC3T3-E1	DUC	DLS, TEM, WB	–	OVX induced osteoporosis in Kunming mice	Four weeks after ovariectomy, 3 mg/kg EVs via tail vein every week for 4 weeks	EVs from middle and mid to late stage of osteoblastogenesis inhibit osteoporosis	[22]
Senescent MLO-Y4 cells	DUC	TEM, NTA, WB	miR-494-3p	SAMP6 mice	100 ng exosomes via tail vein once per week for 4 weeks	Exosomes from senescent MLO-Y4 cells inhibit osteoporosis	[23]
Mice bone marrow macrophages	UC	NTA, TEM, WB	Lnc-LICOE	Rat femoral defect model	50 μg inc-LICOE overexpression iOCL exosomes at defect site thrice a week for 4 and 8 weeks	Lnc-LICOE overexpressing exosomes promoted bone growth	[29]

UC Ultracentrifugation, *DUC* Differential Ultracentrifugation, *TEM* Transmission electron microscopy, *WB* Western blotting *DLS* Dynamic light scattering, *NTA* Nanoparticle tracking analysis, *OVX* ovariectomy

Osteoclasts

Osteoclasts are large multinucleated cells, derived from monocyte/macrophage cells and are responsible for maintaining bone homeostasis via regulating the bone remodeling cycle [81, 82]. However, hyperactivity of osteoclast cells results in bone loss and several bone diseases [83–85]. Due to their significant role in bone remodeling and bone pathologies, numerous studies have looked into the function of EVs from osteoclast cells in the regulation of osteoporosis. It has been observed that EVs extracted from RANK-L-induced RAW 264.7 expressed miR-214, these EVs inhibited osteoblastogenesis in MC3T3-E1 cells, human primary osteoblast cells, and hFOB1.19 cells, however, these effects were diminished by blocking exosomal miR-214 [58]. Expression of miR-214-3p was reported to be elevated in serum of both osteoporotic mice and human osteoporotic patients. Additionally, Osteoclast-derived exosomal miR-214-3p was observed to inhibit osteoblast activity 86. These findings suggest that targeting miR-214 may be a useful therapeutic approach for both promoting bone formation and preventing bone loss [58, 86]. In another study, EVs from RANK-L-induced RAW 264.7 cells were observed to stimulate osteoblastogenesis in the KUSA-O cell line [87]. Exosomes from RANK-L-induced RAW 264.7 cells showed a significant upregulation of miR-23a-5p, and exosomes containing this microRNA inhibited osteoblastogenesis in MC3T3-E1 cells and hFOB 1.19 cells (Fig. 2 and Table 2). These findings suggest that miR-23a-5p regulates osteoblast activity and may therefore be targeted for the treatment of osteoporosis [88]. In a different investigation, the impact of EVs derived from RANK-L-induced osteoclasts (OCL) and lipopolysaccharide (LPS)-induced inflammatory osteoclasts (iOCL) in both *in vitro* and *in vivo* conditions was examined. The findings revealed that iOCL EVs stimulate osteoblastogenesis in MC3T3 cells. The lncRNA lnc-12178 [lncRNA from iOCL-derived exosome (LICOE)], was found to be overexpressed in iOCL exosomes and iOCL exosomes treated osteoblast cells and is involved in promoting osteoblastogenesis in MC3T3 cells. Moreover, it was shown that LICOE overexpressed exosomes enhanced bone growth in a rat model with femoral defect. Thereby suggesting that people with osteoporosis may benefit from treatment using the lnRNA LICOE to promote bone growth [29]. The varied role of osteoclast-derived EVs has been observed in the regulation of osteoporosis, thus, further studies are required to establish the role of osteoclast-derived EVs in the regulation of osteoporosis. Given the observed variations of osteoclasts-derived EVs, additional research is necessary to confirm the diverse function of osteoclasts EVs in the regulation of osteoporosis.

EVs from Other Cells in the Bone Microenvironment

Immune Cells

Immune cells play a very significant role in the pathogenesis of osteoporosis [89–91]. Numerous studies have examined the function of EVs derived from immune cells in preserving bone homeostasis as well as their participation in the pathophysiology of osteoporosis. Ekström et al. found that EVs from LPS-stimulated monocytes from human PBMCs promote osteoblastogenesis in human BMMSCs [92]. In a rat calvaria bone defect model, it was observed that EVs from M0 and M2 cells improved bone healing but M1 EVs impeded bone repair (Fig. 2 and Table 3). In M2 macrophages, miR-378a was found to be highly expressed and involved in the promotion of osteoblastogenesis, whereas miR-155 was found to be significantly elevated in M1 macrophages and involved in the inhibition of osteoblastogenesis [93]. In a different study, exosomes were isolated from M0, M1 and M2 macrophages differentiated from RAW 264.7 cells. Compared to M0 and M1 generated exosomes, it was found that M1 exosomes encouraged the differentiation of mouse BMMSCs towards adipogenesis and osteogenesis [94].

In addition to macrophages, the modulation of bone remodelling has also been examined in relation to other immune cells-derived EVs such as dendritic cells and Treg. Effect of exosome from three distinct subtypes of dendritic cells- Immune regulatory dendritic cell exosomes (regDC EXO), immune stimulatory dendritic cell exosomes (stimDC EXO), and immune null/immature dendritic cell exosomes (iDC EXO) that were differentiated from murine bone marrow cells was evaluated for their bone modulatory role. regDC EXOs loaded with TGFβ-1 and IL-10 were observed to inhibit Th17 cell induction and dendritic cell maturation *in vitro* as well as in an *in vivo* model of murine periodontitis. regDC EXO loaded with TGFβ-1 and IL-10 were also observed to inhibit inflammatory bone loss by promoting the recruitment of Treg cells at the inflamed site and by inhibiting DC maturation and Th17 cell recruitment. While stimDCs had the opposite effect and encouraged bone loss, regDC EXO also reduced osteoclast cell development and hence prevented bone loss [25]. In a study, the role of exosomes from mature DCs (mDC-Exo) and immature DC (imDC-Exo) differentiated from rat bone marrow cells were examined to determine their role in mending bone defects. It was shown that mDC-Exo encouraged the proliferation and *in vitro* differentiation of BM-MSCs into osteoblasts. miR-335, overexpressed in mDC-Exos, inhibited the Hippo signaling pathway to promote osteoblastogenesis in BM-MSCs. In the bone defect model in athymic rats, a biocompatible bone matrix (Orthoss®) embedded with mDC-Exo treated and untreated BM-MSCs was implanted into the femoral defect. It was observed that BM-MSCs treated with mDC exosomes enhanced bone regeneration and that mDC-Exo did not exhibit any of these benefits when miR-335 expression was inhibited. This indicated a potential role for miR-335 in promoting osteoblastogenesis and its potential application as a therapy to promote bone regeneration [95]. Treg exosomes carrying miR-142-3p stimulated osteoblastogenesis in BMMSCs and angiogenesis in HUVECs *in vitro*. In addition, these Treg exosomes promoted fracture repair in a mouse fracture model [24]. Immune cell-derived EVs have been shown to play a crucial role in establishing an osteoporosis therapy strategy, even though there have been fewer studies on immune cells derived EVs in osteoporosis. Given the important role immune cells play in osteoporosis, further research is needed to establish a stronger link between immune cell EVs and osteoporosis to create an immune cell EV-based treatment for osteoporosis.

Table 3 EVs from bone associated cells in osteoporosis therapy

Sample	Isolation method	Characterisation method	Cargo molecule	Model	Dosage	Effect	References
Immune cells							
J774A.1 cells	ExoQuick-TC (System Biosciences) exosome isolation reagent	NTA, TEM, Immunoblotting	miR-378a, miR-155	Calvaria bone defect model in Sprague Dawley rats	Collagen scaffolds containing 8×10^9 EVs/defect from M0/M1/M2 Macrophage were filled in defect for 3 and 6 weeks	miR378a was enriched in M2 EVs and enhanced bone healing while miR-155 was enriched in M1 EVs and inhibited bone healing	[93]
Dendritic cell	UC	NTA, WB	TGFβ-1, IL-10		10^8 particles of regDCs Exosomes/iDCs Exosomes / Stim Exosome at periodontitis site on −2, 0, 2 days in a 9-day peridontitis model	RegDCs exosomes prevented bone loss while Stim DCs exosomes increased bone loss	[25]
Dendritic cells	UC	TEM, NTA, WB	miR-335	Femoral defect model in athymic rat	BMMSCs treated with/without mDC-Exo/mDC-Exo-NC/mDC-Exo-miR-335I were embedded in biocompatible bone matrix (Orthoss®) were filled at defect site for 4, 8 and 12 weeks	miR-335 expressing mDCs enhanced bone regeneration in femoral defect rats	[95]
Tregs	UC	TEM, NTA, WB	miR-142-3p	Femur fracture model in C57BL/6 mice with or without traumatic brain injury	50 μg/ml or 100 μg/ml TregD-Exosomes or 10 μM siRNA negative control, agomiR- 142-3p and antagomiR were injected at fracture site on day 0,4 and 7	Both miR-142-3p containing TregD exosomes and miR-142-3p alone promoted fracture repair	[24]

Endothelial cells							
Bone marrow-derived endothelial progenitor cells	Umibio exosome isolation kit	Immunoblotting, NTA, TEM	–	SIOP model in Kunming (KM) mice	20 mg/kg dexamethasone was injected intramuscularly five times a week for 8 week. After 8 weeks, 50 μg EVs were injected via tail vein once a week for 4 weeks	EPC-EVs prevented SIOP	[26]
Mouse vascular endothelial cells (bEND.3)	DUC	NTA, TEM, WB	miR-155	OVX- induced osteoporotic mice model	Intraperitoneal injection of 100 μg exosome twice a week for 6 weeks	miR-155 containing exosomes prevented osteoporosis	[99]
EPCs from mouse umbilical cord blood	DGUC	DLS, TEM	lncRNA-MALAT-1	Femur fracture model	Intravenous injection of exosome derived from negative control transfected EPCs or from EPCs transfected with lnc-MALAT-1 targeting siRNA	Exosomes from EPCs promote fracture repair	[101]
Muscle cells							
Myogenic C2C12 cells	UC	TEM, NTA, WB	Prrx-2	OVX- induced osteoporotic mice model	4 weeks after ovariectomy, OVX mice were injected twicw a week with Exo-shNC or Exo-shPrrx-2 in femur periosteum	Prrx2 containing myoblast derived exosomes prevented osteoporosis	[105]
C2C12 cells	UC	NTA, WB	–	Femoral defect in streptozotocin induced diabetic mice	10 μg Myo-EVs induced cationized gelatin(CG) hydrogel sheets were placed in femur defect for 4 days	Myo-EVs prevents delayed bone repair in diabetic mice	[30]

UC Ultracentrifugation, *DUC* Differential Ultracentrifugation, *DGUC* Density gradient ultracentrifugation, *TEM* Transmission electron microscopy, *WB* Western blotting, *DLS* Dynamic light scattering, *NTA* Nanoparticle tracking analysis, *OVX* ovariectomy, *SIOP* Steroid induced osteoporosis

Endothelial Cells

Endothelial cells are the blood vessel lining cells derived from endothelial precursor cells (EPCs) and play an important role in angiogenesis [96]. In addition to regulating angiogenesis, endothelial cells also facilitate the regulation of osteoclast and osteoblast cells activity [97]. It has been noted that endothelial cells are important for maintaining bone homeostasis and bone disease treatment [97, 98]. Researchers have also examined endothelial cell EVs function in osteoporosis and discovered that these EVs are significant regulators of osteoporosis. By inhibiting osteoblast ferroptosis, EVs produced from primary bone marrow-derived endothelial progenitor cells (EPC-EVs) reduced steroid-induced osteoporosis *in vivo* [26]. In an additional investigation, exosomes produced from mouse vascular endothelial cells (bEND.3 cells) (EC-Exos) demonstrated superior bone targeting efficacy *in vivo* when compared to exosomes derived from osteoblasts and BMMSCs. In RAW 264.7 cells and bone marrow macrophages (BMMs), EC-exos decreased osteoclastogenesis. EC-Exos also prevented bone loss *in vivo* in ovx C57BL/6 mice. Compared to BMMs and endothelial cells, miR-155 was found to be increased in EC-Exos. It was also observed that miR-155 was involved in suppressing osteoclastogenesis, which suggests that miR-155 may have therapeutic potential in the treatment of osteoporosis [99] (Fig. 2 and Table 3). EPC-EVs from primary bone marrow-derived EPCs from C57BL/6 mice promoted osteoblastogenesis in primary BMMSCs *in vitro* [100]. In a study, EPC exosomes have also been shown to stimulate osteoclastogenesis in BMM. LncRNA-MALAT1 was found to be increased in EPC exosomes and to target miR-124 activity to stimulate osteoclastogenesis *in vitro*. Additionally, it was found that EPC exosomes improved bone regeneration in a fracture model in mice, indicating a possible therapeutic use for EPC exosomes in fracture healing [101]. Owing to the important function endothelial cell EVs play in controlling bone cells and *in vivo* models of osteoporosis, more research may be necessary to confirm the significance of endothelial cell EVs and to expedite the development of EV-based osteoporosis therapies.

Muscle-Derived EVs

The interaction of muscles and bones enables all bodily movements [102] & loss of muscles is associated with bone loss diseases like osteoporosis [103]. Consequently, a growing body of research has assessed the function of EVs produced from muscle cells in osteoporosis. In a study, muscle derived EVs from culture supernatants of normal skeletal muscles (Mu-EVs) from hind limbs of C57BL/6 mice were observed to promote osteogenesis in BMMSCs and inhibited the differentiation and maturation of osteoclast cells (Fig. 2 and Table 3) while EVs from atrophic muscles inhibited osteoblastogenesis and promoted osteoclastogenesis. Mu-EVs enhanced bone formation in the *in vivo* model of disuse-induced osteoporosis, indicating a potential therapeutic role for them in treating osteoporosis [104]. Exosomes derived from C2C12 cells that underwent myoblast differentiation have been shown to augment osteogenesis in BMMSCs. Elevated Prrx2 was associated with increased osteoblastogenesis in both *in vivo* and *in vitro* conditions raising the possibility that Prrx2 could be a target for future therapy [105]. In a different study on RAW264.7 cells, EVs from C2C12 cells undergoing myoblast differentiation suppressed osteoclastogenesis [106]. The same group found that elevated miR-196-5p was involved in and suppressing osteoclastogenesis in RAW264.7 cells and enhancing osteoblastogenesis in ST2 and MC3T3 cells in another investigation [107]. This research group also looked at muscle-derived EVs, or Myo-EVs, in secondary osteoporosis mice, which are diabetic mice treated with streptozotocin. In diabetic mice, local delivery of Myo-EVs at the site of bone damage increased osteoblastogenesis and improved bone healing [30]. Exosomes derived from C2C12 myoblasts stimulated osteoblastogenesis in MC3T3 cells via upregulated miR-27a-3p expression. EVs produced from skeletal muscle also improved glycolysis, which in turn enhanced osteoblastogenesis in BMMSCs, and prevented bone loss in disuse osteoporotic mice after muscle atrophy [108]. While several studies have assessed the function of muscle EVs in bone homeostasis and osteoporosis, more research is necessary to create a treatment strategy utilizing muscle cell EVs for osteoporosis.

EVs from Other Organisms

Bacteria-Derived EVs

The relationship between bone health and gut microbiota and other inflammatory microorganisms has been documented in numerous studies [109–111]. Studies have shown that inflammatory disorders like osteoporosis affect the gut microbiota [112]. Treating osteoporosis with specific probiotics, such as *Lactobacillus rhamnosus* [113], *Bifidobacterium longum* [114], *Prevotella histicola* [115], and others, has been observed to be an effective strategy. Nevertheless, the function of bacteria or gut microbiota-derived EVs in controlling and treating osteoporosis has not been thoroughly studied. It was observed that there is a reduction of *Akkermanasia mucinniphila* (Akk) bacteria in ovariectomy-induced osteoporotic mice and administering Akk EVs to ovx mice reduced bone loss by inducing osteogenesis and inhibiting osteoclastogenesis [116] (Fig. 2 and Table 4). In addition to probiotic bacteria, pathogenic bacteria *Proteus mirabilus* outer membrane vesicles suppressed osteoclastogenesis *in vitro* by causing osteoclasts to undergo mitochondria-dependent apoptosis. Additionally, it prevented bone loss *in vivo* in mice with ovariectomy-induced osteoporosis and collagen-induced arthritis models [117]. Additionally, microorganisms have been utilized to create engineered EVs, which are used to treat osteoporosis. In a study, Escherichia coli Nissle 1917 (ECN) genetically modified to overexpress hCXCR4 on the surface of BEVs and were loaded with SOST siRNA, resulting in BEVs-hCXCR4-SOST siRNA (BEVs-CSs). These EVs reversed osteoporosis in ovx mice and demonstrated excellent bone-tracking ability in addition to promoting osteogenesis *in vitro* [31].

Table 4 EVs from bacteria and plants in osteoporosis therapy

Sample/ source	Isolation method	Characterisation method	Cargo molecule	Model	Dosage	Effect	References
Bacteria derived EVs							
Akkermansia muciniphila (Akk)	UF/ Optiprep DGUC	TEM, DLS	–	OVX- induced osteoporotic mice model	OVX mice were orally administered with 1.2×10^{10} vesicles or intravenously injected with 3×10^{9} vesicles from CGM/EGM /Akk-EVs twice a week for 2 months	CGM-EVs and Akk-EVs prevented osteoporosis	[116]
Proteus mirabilis	UC	NTA, TEM	–	OVX- induced osteoporotic mice model	Intra-articular injection with 5×10^{8} particles/ml once a week for 2 months	OMVs from *Proteus mirabilis* prevented bone loss	[117]
Plant derived EVs							
Pueraria lobata	UC	TEM, NTA		OVX- induced and TMAO induced osteoporotic mice model	OVX mice and TMAO treated mice were intraperitoneally injected with 10 mg/kg PELNs	PELN EVs prevented bone loss in osteoporotic mice	[128]

UC Ultracentrifugation, *DUC* Differential Ultracentrifugation, *DGUC* Density gradient ultracentrifugation, *TEM* Transmission electron microscopy, *DLS* Dynamic light scattering, *NTA* Nanoparticle tracking analysis, *OVX* ovariectomy, *CGM* Child gut microbiota, *EGM* Elderly gut microbiota, *PELNs Pueraria lobata* derived exosomes like nanovesicles, *TMAO* trimethylamine-N-oxide

Given the advantages of probiotics in treating osteoporosis, more research on the use of EVs produced from the gut microbiota in osteoporosis treatment is necessary. Furthermore, because bacteria can be grown on a large scale, they may offer a more effective method of separating enough EVs for therapy; the only thing to watch out for is controlling growth conditions to prevent batch-to-batch variations.

Plant-Derived EVs

The use of plant-based medications to treat illness has been well-known since ancient times [118]. It is well-recognized that plant metabolites, roots, and leaves can treat several illnesses, including human immunodeficiency virus (HIV) [119], cancer [120], immunological problems [121, 138], and osteoporosis [122]. Natural plant-based products, however, have problems with metabolism and bioavailability [123, 124]. More recent research employs plant-derived EVs to create a successful medicinal approach. Plant-derived EVs have demonstrated encouraging outcomes in the treatment of various diseases via their immunomodulatory, antioxidant, and antibacterial properties [125]. Plum-derived EVs, were the first known plant-derived EVs to be studied for OP treatment. These EVs inhibited osteoclastogenesis and increased osteoblastogenesis in the MC3T3-E1 cells and osteoblast cells derived from mouse bone marrow [124]. It has also been reported that EVs produced from apples promote osteoblastogenesis in MC3T3-E1 cells [136]. In a study by Seo et al., ginseng-derived EVs reduced osteoclastogenesis in primary BMMs and the LPS-induced bone loss mouse model, indicating a potential anti-osteoporotic role for these EVs [137]. In addition, Yam-derived EVs increased osteoblastogenesis in MC3T3 and murine bone marrow cells and improved bone characteristics in an ovx-induced osteoporotic mouse model [127]. Exosomes from *Pueraria lobata* demonstrated increased osteoblastogenesis in primary BMMSCs and ovx-induced osteoporotic SD rats. Additionally, *Pueraria lobata* exosomes degraded trimethylene-N-oxide (TMAO), a gut metabolite linked to the advancement of osteoporosis [128]. Given the encouraging outcomes seen when employing EVs obtained from plants to treat osteoporosis, further research in this area is required to create a plant EV-based osteoporosis treatment.

5 Limitations in the Clinical Translation of EVs

Research on EVs in osteoporosis and other disorders has demonstrated their significant potential of EVs in disease diagnosis, treatment, and drug delivery. Despite promising preclinical findings, only a small fraction of studies has transitioned into clinical trials, primarily due to various limitations associated with the current EV research methodologies [129]. The primary issue impeding the clinical translation of EVs is the lack of good manufacturing practice (GMP) standards for EV isolation [130, 131]. The first step in the EV isolation is the determination of cell type for the

EV isolation [132]. EVs imitate the phenotypic condition of the parent cell and so the EVs derived from the same cell type may exhibit paradoxical effects due to variations in culture conditions or source cell storage conditions [130].

This leads to contradictions among the results of different laboratories, necessitating the standardization of GMP-grade experimental protocols for EV studies. Furthermore, the lack of standardized isolation and quantification techniques contributes to inconsistencies in EV research [39]. The choice of isolation technique impacts EV yield and purity, highlighting the need for standardized protocols that ensure high-purity isolation while maintaining sterility to facilitate clinical translation [131]. Similarly, for the use of EVs as a therapeutic agent it is necessary to quantify EVs to determine effective doses of EVs. Different quantification techniques are available based on particle number or protein, RNA, or lipid content evaluation of EVs and there is a requirement for a standard quantification technique to allow the evaluation of effective dose [129]. The scalability of EV production is another limitation, as laboratory-scale experiments often yield insufficient quantities of EVs for clinical applications [131]. Addressing this challenge requires the development of scalable production methods to generate high yields of EVs suitable for clinical use. Storage conditions also pose challenges, as different storage conditions may affect the stability and biological activity of EVs [39, 133]. Establishing optimal storage conditions that preserve EV structural integrity and activity is crucial for their clinical translation. Additionally, native EVs are reported to have poor drug-loading efficiency and targeting, this can be addressed through engineering EVs to improve their drug-loading efficiency and enhance their specificity for target sites [27, 39].

However, the use of both native EVs and engineered EVs needs to be evaluated further for their efficacy and safety. Safety evaluation is paramount before proceeding to clinical trials, as few studies have reported that EVs may exhibit cytotoxic, immunogenic, or tumorigenic potential [129]. Comprehensive pharmacokinetic studies in relevant animal models are essential to assess the safety and efficacy of EVs *in vivo* [129, 136]. In conclusion, while EVs hold tremendous promise for clinical applications in diseases, overcoming the challenges associated with their clinical translation requires coordinated efforts to standardize EV isolation, quantification, and storage, along with rigorous safety evaluation and efficacy assessment in preclinical models. Addressing these hurdles will pave the way for the successful clinical application of EV-based therapies.

6 Conclusion and Future Perspective

This chapter highlights the critical role of EVs in the context of osteoporosis management. Through a comprehensive review of recent research, we have elucidated the potential of EVs as diagnostic biomarkers for osteoporosis. Moreover, the therapeutic potential of EVs in mitigating osteoporosis with research on both native and engineered EVs yield encouraging results in preclinical studies. Future research should concentrate on several important topics. Firstly, there is a need for more comprehensive research to clarify the precise molecular cargo within EVs that contribute to the pathophysiology of osteoporosis. This could lead to the discovery of new biomarkers and potential therapeutic targets. Furthermore, to guarantee the reproducibility and clinical translation of EV-based treatments, standardized protocols for EV isolation and characterization techniques should be developed [39]. Moreover, large-scale clinical trials are necessary to assess the safety, efficacy and long-term effects of EV-based therapies in patients. Advancements in bioengineering techniques hold the potential to enable the development and refinement of EV-based therapeutics [137]. Overall, research on the theragnostic potential of EVs in osteoporosis is still in the early stages but holds significant potential for the development of management and treatment strategies for osteoporosis.

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Nanocellulose Scaffolds for Skin Tissue Engineering

Twinkle Gupta and Shilpa Sharma

1 Introduction

Skin is the largest and most important organ in the human body, comprising about 7% of adult body weight and 2 m^2 of surface area. Among its numerous functions is acting as a barrier to safeguard against harmful external substances, toxic agents, microbial pathogens, UV light and mechanical injury [1, 2]. Its importance becomes clear when deep burns or traumas that involve multiple regions of the human body cause significant skin loss. Delay in the treatment can result in development of chronic wounds, fluid and electrolyte imbalance, poor heat regulation, and sepsis, all of which can ultimately result in death [3]. Skin grafts can be a choice; however, donors are not always available [4]. Employing allografts leads to the possibility of immunological rejection and viral disease transmission, in addition to difficulties in integrating the transplant into the surrounding tissue, and donor site morbidity.

A complete bioengineered skin that replicates intricate skin tissue in both shape and function is still challenging. Due to high demand for skin replacements, researchers are motivated to develop a perfect skin substitute that mimics natural skin in order to promote stable wound healing and regeneration [5]. Tissue engineering is the process of repairing and rebuilding various tissues and organs, including skin, muscles, blood vessels, bones, ligaments, intervertebral discs, etc. Skin tissue regeneration is a rapidly expanding area of biomedical research with an aim to return skin to its natural form. It focuses on creating novel treatments for skin-related disorders such as burn injuries and wound healing [1]. Healing a skin wound is a complex and dynamic process that involves interaction of cells, growth factors, and cytokines to heal the lesion. Skin tissue engineering requires not only

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appropriate cells and growth factors but also a synthetic extracellular matrix (ECM) that helps in the proliferation and differentiation of cells. In order to promote cellular adhesion, migration, proliferation, and ECM synthesis and aid in tissue integration and regeneration *in vivo*, biomimetic scaffold design attempts to mimic native tissue architecture. Multiple cell types regulate this process by secreting a variety of growth factors, cytokines, and chemokines [6]. Aspects like scaffold's porosity, pore size, and pore structure are crucial and need to be taken into account during the scaffold design process. Furthermore, scaffold must be biocompatible i.e., non-immunogenic and non-thrombogenic, as well as resistant to infection [5]. A variety of biomaterials including synthetic polymers and natural materials like silk fibroin, collagen, fibrin, alginate, hyaluronic acid, cellulose, and proteoglycans have been explored as scaffolds to support skin regeneration. In this context, natural materials have the advantages of low toxicity and a less chronic inflammatory response [3]. Nanocellulose (NC) is a form of cellulose in nano-range in at least one dimension (1–100 nm) and several meters long. The specific characteristics of nanocellulose are high surface area, porosity, excellent mechanical stability, electrical conductivity, lighter mass, low immunogenicity and biodegradability. It can be easily chemically modified to alter its characteristics which enables its use for various applications.

2 Cellulose and Its Sources

Cellulose, a linear polymer of glucose, is the most abundant biopolymer on earth. In recent years, nanocellulose extracted from native cellulose has gained considerable interest as a new type of bio-scaffold in tissue engineering owing to its excellent physical and mechanical properties, tailorable surface chemistry as well as biocompatibility, biodegradability and low toxicity [7]. Its substantial specific surface area promotes more cell adhesion and growth. Cellulose can be obtained from various sources such as wood-based cellulose, plant-based cellulose, bacteria-based cellulose; algae-based cellulose, and tunicate-based cellulose (Fig. 1). Woods and plants are the primary sources of cellulose, which have been extensively used in industry due to cost- effectiveness [8]. Some typical sources of cellulose in wood and plants are hardwood trees, soft wood trees, cotton, bamboo, flax, hemp, jute, kenaf, agave, sugarcane, palm leaves, corn, etc. In wood, cellulose is present along with hemicellulose and lignin. Cellulose fibres, which are interwoven and give wood its extraordinary strength and stiffness, make up the majority of wood.

Bacterial cellulose (BC) is produced by specific bacteria, primarily those belonging to the genera *Acetobacter*, *Agrobacterium and Sarcinaventriculi*, etc. It differs from plant cellulose in that it has higher purity, greater strength, moldability, and a greater capacity to store water [9]. BC has been found to be 97% pure with respect to plant-based cellulose, because of absence of other polymers like lignin or hemicellulose. Consequently, the process of extracting and refining BC is relatively straightforward and does not require extensive chemicals or other forms of

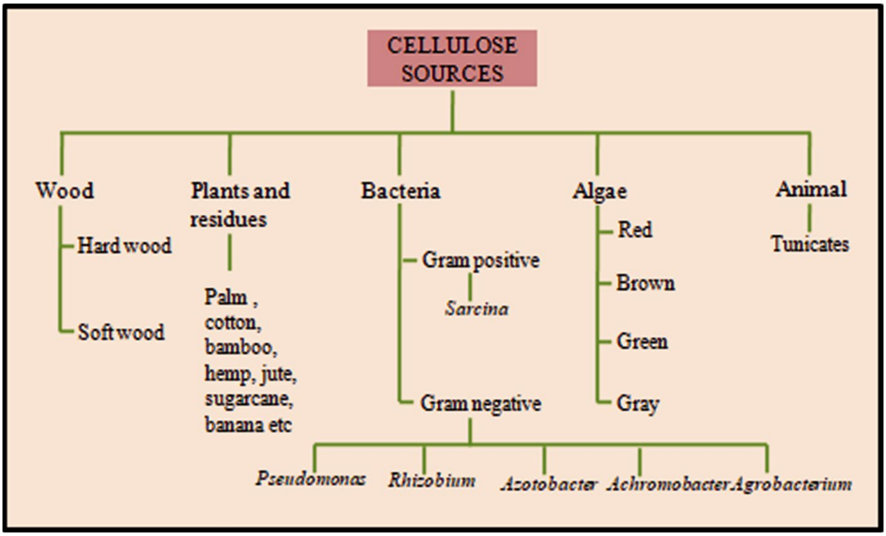
CELLULOSE SOURCES
Wood
Hard wood
Soft wood
Plants and residues
Palm, cotton, bamboo, hemp, jute, sugarcane, banana etc
Bacteria
Gram positive
Sarcina
Gram negative
Pseudomonas
Rhizobium
Azotobacter
Achromobacter
Agrobacterium
Algae
Red
Brown
Green
Gray
Animal
Tunicates

Fig. 1 Sources of cellulose

treatment, which distinguishes it from wood and plant cellulose. The most widely employed carbon sources for the synthesis of BC are glucose and sucrose, while fructose, maltose, xylose, starch, and glycerol have also been used. Due to exceptional mechanical qualities, high water holding capacity, and exceptional suspension stability, BC has attracted significant attention in biomedical field and industries [10]. Other significant sources of cellulose include a variety of algae, such as brown species *Posidoniaoceanica*, *Laminariahyperborea, Alariaesculenta,* green species *Microspora*, *Chlorophyceae, Cladophora*, and red species *Rhodella maculate, Gelidiumelegans*, *Gracilaria*, the cell walls of which are largely made of cellulose [11]. By improving growth conditions and extraction techniques, the yield of cellulose in algal biomass can be raised [12]. Cellulose extracted from algae has high crystallinity, low moisture adsorption capacity, high porosity in the mesoporous range, and high surface area [13]. Algal cellulose has a lot of potential as a renewable and sustainable supply of cellulose for a variety of uses, but further studies are needed to improve its production and processing. Tunicate cellulose is present in the tunic i.e. extra-protective covering of marine animals or tunicates such as *Ascidia* sp., *Cionaintestinalis*, *Styelaplicata*, *Styelaclava*, *Halocynthiaroretzi*, Halocynthiapapillosa [14]. In terms of mechanical characteristics, thermal stability, molecular weight, water holding capacity, and permeability, tunicate cellulose is superior to plant-based cellulose. Additionally, the crystallinity index of tunicate cellulose is higher than that of plant-based and BC and comparable to that of algal cellulose [14]. Its pronounced crystallinity hinders its solubility in water, limiting its practical applications to a significant extent [15].

2.1 Nanocellulose

Nanocellulose is defined as the natural fiber extracted from cellulose molecules with at least one dimension in the nanoscale (1–100 nm). Nanocellulose possess attributes like chemical inertness, stiffness, high strength, low coefficient of thermal expansion, low density, dimensional stability, and the capacity to alter its surface chemistry in addition to being abundant, biodegradable, and renewable [16]. Nanocellulose is categorised into three main types based on synthesis conditions that determine its dimensions, composition, and properties: cellulose nanofibers (CNFs), cellulose nanocrystals (CNCs), and bacterial nanocellulose (BNC). Although all three types are similar in chemical composition, they are different in morphology, size, and some properties due to different sources and extraction methods. The term "nanofibers" is typically used to refer to cellulose fibrous structures that possess diameter range between 5 and 30 nm and length in several microns. Although, they are strongly twisted submicron-scale fibres, the name "nanofibers" has gained popularity for describing them [17]. CNFs consist of highly crystalline microfibrils with distinct characteristics and sizes. CNCs are cellulose-derived charged nanoparticles with a high aspect ratio. CNCs are often shaped like rods, ribbons, or whiskers, with lengths ranging from tens of nanometres to several microns and widths ranging from 3 to 50 nm. BNC is a biopolymer produced by a diverse range of bacteria (*Sarcinaventriculi*, *Azotobacter*, *Acetobacter*, and *Pseudomonas*) that is natural and chemically pure. Its production doesn't involve polymerization or the merging of minute particles. Therefore, the typical shape of BNC is due to combination of glucose chains which leads to the formation of the network of cellulose nanofibrils structures [18]. It is a highly biocompatible material that has been employed in a variety of biomedical applications such as drug delivery carriers, blood vessels, prosthetic skin, and wound dressings [19]. Because of its unique self- assembling capabilities, BNC is suitable for a wide range of biological applications (Table 1).

3 Preparation Strategy of Nanocellulose

Nanocellulose is isolated from native cellulose which usually involves two main steps. The first stage is pretreatment of feedstocks to produce pure cellulose while in the second stage, cellulose is converted into nanocellulose. The goal of pretreatments and mechanical methods is to break the lignocellulosic complex and solubilize non-cellulosic components (lignin and hemicellulose) while preserving the materials for future valorization, reducing cellulose crystallinity, and increasing porosity for the subsequent depolymerization process [28]. There are several traditional methods for nanocellulose preparation. However, the choice of method is dependent on the type of nanocellulose (CNF or CNC) being prepared.

Table 1 Types of Nanocellulose with methods and dimensions of nanostructures.

Nanocellulose Structure	Source	Method	Dimensions and size	References
Cellulose Nanofibers	Corn Husk Sugarcane bagasse	TEMPO oxidation Enzymatic hydrolysis, TEMPO Oxidation	5–8 nm 60–1820 nm	[20] [21]
Nanocrystals	Cotton Bleached aspen Kraft pulp	Acid hydrolysis, Ultrasound Acid hydrolysis	221 nm particle size 3.38 diameter	[22] [23]
Nanowhiskers	Bleached commercial softwood Kraft pulp	Sulfuric acid hydrolysis	150–300 nm in length and 4–8 nm in diameter.	[24]
Nanorods	Cellulose fibers Sodium carboxy-methyl sodium	Mechanical stirring In-situ polymerization	60–570 nm in diameter Diameter 100 nm	[25] [26]
Nanoplatelets	Agave	Lyophilization process, mechanically milling	Thickness 70–80 nm	[27]

3.1 Pretreatment Methods

In pretreatment processes, various extractives, free sugar, tannins, lignin, hemicellulose, flavonoids are partially or totally eliminated from the biomass. Also, these techniques help in to reducing energy consumption and boost the level of nanofiber production [29]. A common and effective technique for obtaining hemicellulose as a water-soluble fraction is dilute acid hydrolysis method. Lignin dissolves more readily with alkali treatment; however, reaction conditions must be carefully monitored to prevent alkaline hydrolysis of cellulose.

3.2 Production Methods of Nanocellulose

Formation of nano-fibrillation of cellulose is achieved by chemical hydrolysis, enzyme hydrolysis, and mechanical methods (Fig. 2). These methods affect the morphology and size of the final products.

TEMPO-Oxidation Method

Oxidation is a chemical process that breaks down cellulose and modifies functional groups on the fibril surface.2, 2, 6, 6-tetramethylpiperidine 1-oxyl (TEMPO) mediated oxidation is primarily used to decrease the energy needed for mechanical disintegration by decreasing the negative or positive charge on the fibre surfaces and enhancing the stability of the colloidal suspension of generated nanocellulose [30].

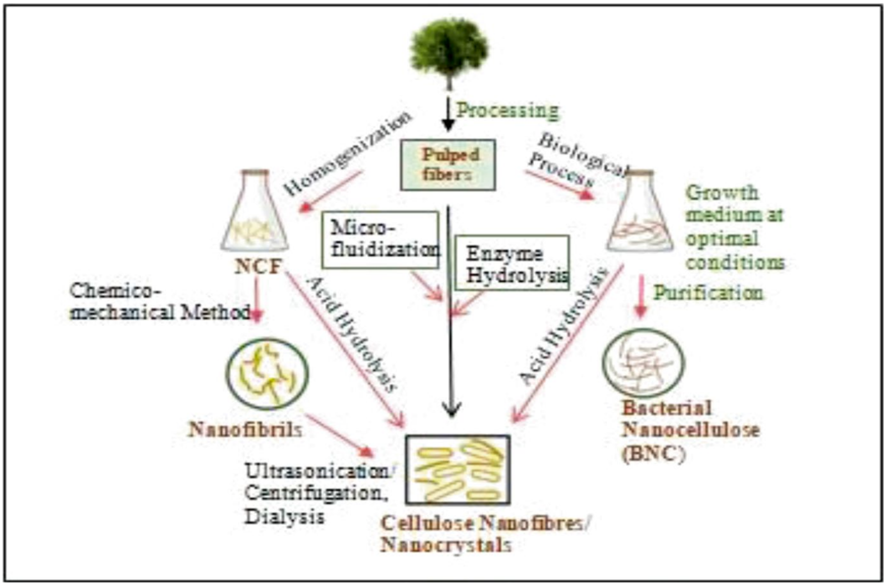
Processing
Homogenization
Pulped fibers
Biological Process
Growth medium at optimal conditions
NCF
Micro-fluidization
Enzyme Hydrolysis
Chemico-mechanical Method
Acid Hydrolysis
Acid Hydrolysis
Purification
Nanofibrils
Bacterial Nanocellulose (BNC)
Ultrasonication/ Centrifugation, Dialysis
Cellulose Nanofibres/ Nanocrystals

Fig. 2 Various methods of nanocellulose synthesis

Under aqueous conditions, TEMPO is used for the catalytic and selective oxidation of primary hydroxyl groups at the C_6 position of cellulose that creates carboxylate groups which are imperative for dispersion and stability of nanocellulose suspensions [31]. This process is carried under normal pressure at room temperature with mild reaction conditions, which results in minimal loss of materials. Additionally, the crystallinity of nanocellulose does not get altered significantly. However, it has some disadvantages: use of hazardous chemicals, difficult to recycle and limited oxidation position [32].

Acid/Alkaline Hydrolysis

In acid hydrolysis, β-1-4 glycosidic linkages in cellulose are disrupted in the presence of mineral acid (50–72% sulphuric acid). The low density of amorphous regions in natural cellulose makes them more acid-accessible and vulnerable to hydrolytic action of acid than crystalline [33]. This process is commonly used to obtain CNCs mostly of 4–70 nm in width, 100–6000 nm in length. Figure 3 shows crystalline area of nanocrystalline cellulose after hydrolysis of the amorphous region of the cellulose chain. Alkaline hydrolysis of cellulose occurs in the presence of alkali, which serves as a swelling agent thereby increasing internal surface area, decreasing the degree of polymerization and crystallinity of cellulose. It also dissolves hemicelluloses and disrupt the lignin structure by breaking glycosidic ether bond [34]. The disadvantage of these processes is that they require lot of water

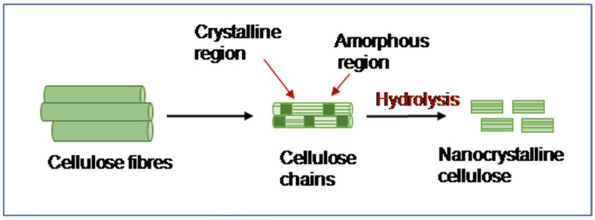

Fig. 3 Schematic of nanocrystalline cellulose derived from cellulose chains by hydrolyzing the amorphous portion and leaving the crystalline region

consumption to recover neutrality. Also, long duration of acid hydrolysis minimizes the thermal stability and degree of crystallinity.

Ionic Liquids (ILs)

They are organic salts with low vapour pressure and have unique characteristics like non-flammability, electrical conductivity, thermal and chemical stability. Under relatively mild conditions, ILs can dissolve cellulose; they are reusable liquid salts at room temperature and often consist of inorganic anions and organic cations [35]. ILs break the cellulose hydrogen-linked structure by developing another hydrogen bond in a 1:1 ratio between the IL anion and cellulose (sugar hydroxyl protons), thereby reducing cellulose compactness and making it more amorphous and prone to depolymerization [36]. The most common ILs for dissolving cellulose are1-butyl-3-methylimidazole chloride, 1-allyl-3-methylimidazole chloride, and 1-ethyl-3-methylimidazole chloride.

Enzyme Hydrolysis

The enzyme hydrolysis is an emerging method to obtain nanocellulose from lignocellulosic biomass which involves different steps. The first step involves pretreatment process to get pure cellulose by eliminating lignin, hemicellulose and other extractives. Second step involves hydrolysis of cellulose, using different combinations and ratios of cellulases like endoglucanases, exoglucanases, β-glucosidases, etc. [37]. The cellulosic fibres cannot be broken down by a single specific enzyme since cellulose is made up of numerous different chemical components [38]. Endoglucanase randomly break cellulose chains into shorter length, exoglucanase (cellobiohydrolase) attacks on non-reducing ends of crystalline cellulose to generate cellobiose and β glucanases that attack the cellobiose to produce glucose. The third step involves mechanical treatment like homogenization of enzymatically treated fibres using sonication, ultrafine grinder, microfluidizer, etc. [38]. The enzymatic hydrolysis method is sustainable and environment friendly. Biological treatment with enzymes requires mild conditions; however, a long operating period is required.

Mechanical Process

It involves mechanically breaking down cellulose strands into smaller particles. Cellulosic fibres can be converted into nanocellulose via a variety of mechanical processes, including homogenization [39] micro fluidization [40], grinding [41], cryo-crushing [42] and high-intensity ultrasonication [43].

Homogenization

High-pressure homogenizers (HPH) are effective for delaminating cellulose fibre cell walls and liberating nano sized fibrils a large-scale for industrial applications. Pre-prepared cellulose is frequently driven through a valve at extremely high pressure, causing the cellulose fibres to delaminate along their longitudinal axis, yielding nano-fibrillated cellulose. After several repetitions of homogenization, the particle sizes would typically be less than 50 nm, several micrometers in length, and have a reasonably narrow particle size distribution. The HPH is a quick and easy process to set up with no solvent requirement and there is no production of waste water. However, using mechanical processing alone may have the disadvantage of high energy consumption, disturbing crystallinity of nanocellulose and high operating cost.

(a) **Grinding:** Nanocellulose can be produced easily and effectively through grinding in which cellulose pulp is passed through couple of stones where one stone is fixed and other is rotatable leading to disruption of cellulose and hydrogen bonds by shear forces, resulting in cellulose nanofibers production. This method can be combined with various pretreatment procedures such as disk refining, ultrafine grinding, ball milling, etc. The disadvantages of this process are high cost, low recovery and high energy consumption and reduction of nanocellulose's crystallinity.
(b) **Microfluidization:** In this process, cellulose is fed into the microfluidizer where it is subjected to strong shear and pressure forces, producing nanocellulose [41]. Microfluidizer converts cellulose fibers into nanocellulose fibers by using high-pressure fluid streams. Homogenization is comparable to microfluidization, but because there are no moving elements, there is less chance of obstruction. In the interaction chamber, the shear force breaks down the fibers by acting on the channel wall and collision current. The process of creating CNF involves repeatedly homogenizing the fiber-water solution, which uses a lot of energy.
(c) **Cryo-crushing:** It is a non-hazardous method for producing nanocellulose [42]. In this technique, cellulose fibers are soaked in water and then immersed

in liquid nitrogen. The frozen water-soaked fibres are then broken down into tiny particles using strong shear forces. The high forces result in the rupture of cell wall of cellulosic particles due to high pressure created by ice crystals. This process has drawbacks of like high energy consumption, high cost, low recovery and less uniform nanocellulose.

(d) **High-intensity ultrasonication:** High-intensity sonication (HIU) uses ultrasonic waves to break down cellulose fibers into smaller fragments. This process generates mechanical oscillating power, which causes small gas bubbles to develop, expand, and burst when the liquid molecules absorb ultrasonic energy. Because of the intense cavitational high shear forces, ultrasonic technology outperforms the traditional nanocellulose manufacturing methods [43]. The NC obtained via HIU method has increased crystallinity index, thermal stability, viscosity and reduction in cellulose chain lengths. This method can be followed by several methods like acid hydrolysis, enzymatic method to get more nanofibrillated fibers. Although, this method uses high energy consumption and high cost, it is eco-friendly and more efficient method of nanocellulose production.

4 Properties of Nanocellulose

Nanocellulose is an intriguing material due to its attractive optical, rheological, and adjustable surface chemistry properties, as well as high mechanical strength, thermal stability, high aspect ratio, biodegradability, and renewable nature. Currently, nanocellulose can be produced on an industrial scale in tons per day. It has drawn a lot of attention because of its unique characteristics and numerous potential uses in a variety of industries, including food packaging, antimicrobial films, textiles, cosmetics, and biomedical devices [44].

4.1 Structure Chemistry

Cellulose is a semi-crystalline polysaccharide with D-glucopyranose units connected by β 1-4glycosidic linkages [44]. Due to its high polarity and capacity to form potent hydrogen bonds, it makes a superb high-strength support and carrier material. The stabilization energy of cellulose for each hydrogen bond is thought to be quite low since the hydrogen bond of cellulose is primarily electrostatic [45]. The use of dynamic nuclear polarization (DNP)-enhanced NMR spectroscopy has been an emerging tool to understand the surface chemistry of cellulose. However, there is still lack of comprehensive structural and chemical characterization of nanocellulose's surface chemistry, particularly for very low levels of functionalization (<1 wt.%). Furthermore, surface modification of nanocellulose results in high tensile strength, tensile modulus, optical transparency, and thermal stability. It has been demonstrated that adding hydrophobic polymers to nanocellulose boosts its stability by modifying its surface which increases its wettability in the oil phase and decreases interfacial tension [46]. These tailorable features of nanocellulose makes it a suitable material as a tissue bio-scaffold in skin tissue engineering, promoting binding and proliferation of cells, in addition to wound repair and regeneration of skin.

4.2 Mechanical Properties

The reinforcement of biopolymers with nanocellulose results in high mechanical strength and durability. Nanocellulose has an average modulus of 100 GPa, which is significantly higher than that of the cellulose. Nanocellulose-based materials and their composites have been actively used for the creation of high-performance mechanical and functional materials [13]. The nanocellulose, even at low concentrations acts as a filler material that enhances mechanical (tensile strength), thermal, optical, and chemical features (high specific surface area, -OH groups) of the nanocomposite. Nanocellulose and other polymer matrices are linked with strong intermolecular connections, such as covalent bonds, vander waals forces, mechanical interlocking and entanglement which is responsible for high strength and stiffness. The crystallinity index which affects the mechanical and physical properties of nanocellulose is analysed using X-ray diffractometer (XRD). The crystallinity index of CNCs is comparatively higher (54–88%) then cellulose fibrils which enhances mechanical and thermal properties. However, crystallinity, and mechanical properties of nanocellulose rely on the source of raw material and its extraction process, which will determine its applicability. There was a significant decrease in crystallinity of nanocellulose due to severe mechanical processes of depolymerization of cellulose while enzymatic treatment improved the crystallinity and crystalline index (45–90%) [47].

4.3 Biocompatibility and Non Toxicity

Biocompatibility describes a material or substance's capacity to interact safely and favourably with biological systems, such as living things or tissues. In essence, a biocompatible substance is well-tolerated by the body and does not significantly elicit an immune response or cause toxicity when it comes in touch with biological systems. Biocompatible materials are essential in tissue engineering, as they do not injure or adversely affect the host organism or tissue [48, 49]. The toxicological studies on nanocellulose materials are extremely confusing, contradictory, and inconsistent. Currently, majority of researchers concur nanocellulose to be nontoxic as several studies have shown no or little effect of nanocellulose materials on the skin cells and other cell types, making it appropriate for use in tissue engineering where cell viability is important. Few studies indicate that the toxicity levels of nanocellulose may vary depending on the synthesis method. Nanocellulose extracted using enzyme hydrolysis method has been reported to be non-cytotoxic at any concentration [50]. CNFs with high aspect ratios, produced by mechanical homogenization were found to be more toxic than CNF or CNC produced by chemical synthesis method [51]. Although current studies on nanocellulose have not revealed any significant environmental or biological issues, more in-depth study and a thorough evaluation of toxicity of nanocellulose is still required. Long-term *in vivo* toxicity evaluation of nanocellulose should be the main focus. It is also critical to look into not just the toxicity of nanocellulose itself but also any potential toxicity consequences brought on by its incorporation. Comprehensive examination is still necessary despite the general absence of severe cytotoxicity seen in many reports employing nanocellulose-based products [52, 53]. Owing to low toxicity, renewability, good biocompatibility, and excellent physical properties, nanocellulose is widely used in the biomedical field [54]. Nanocellulose based dressings adhere to the wound till the skin heals and may be easily removed In recent years, researchers have also explored other uses of nanocellulose in medicine, including blood vessel replacement, soft tissue implants, and drug delivery to specific cells.

5 Applications of Nanocellulose in Skin Tissue Engineering

Nanocellulose has received widespread attention for potential applications in skin tissue engineering and wound healing. It is able to preserve moisture in damaged skin and absorb exudates from wounds owing to its strong mechanical strength and high water-absorbing ability [55]. It has a nanoscale shape that closely mimics native extracellular matrix's nanoscale design, making it an ideal substrate for skin cells to adhere to and grow on. Fabrication of 3D scaffolds for tissue repair involves several crucial phases, including designing and manufacturing. It is imperative that scaffolds for skin tissue engineering possess proper physical and mechanical properties to stimulate optimal cell growth conditions for cell adhesion, proliferation, and differentiation (Fig. 4). Till date, several manufacturing methods have been developed with different levels of success to create 3D scaffolds for skin tissue engineering applications [56].

In this context, 3D scaffolds containing CNFs or CNCs have been developed using techniques like solvent casting/ particle leaching, freeze drying, phase separation method, gas foaming, sol gel, electrospinning, 3D printing, bio-printing, direct cell writing, fused deposition modelling, stereo lithography, etc. (Fig. 5). The production process and the materials used determine scaffold's properties. The form of material, its structure, molecular weight, surface chemistry, surface charge, solubility, lubricity, degradation mechanism, water absorption capacity, water resistivity and intended use are crucial factors to consider while fabricating scaffold for skin tissue engineering applications [57]. Materials having porous and linked structures are thought to be effective supports for encouraging wound healing because they may deliver antibiotics or other drugs to the desired place. When mixed with

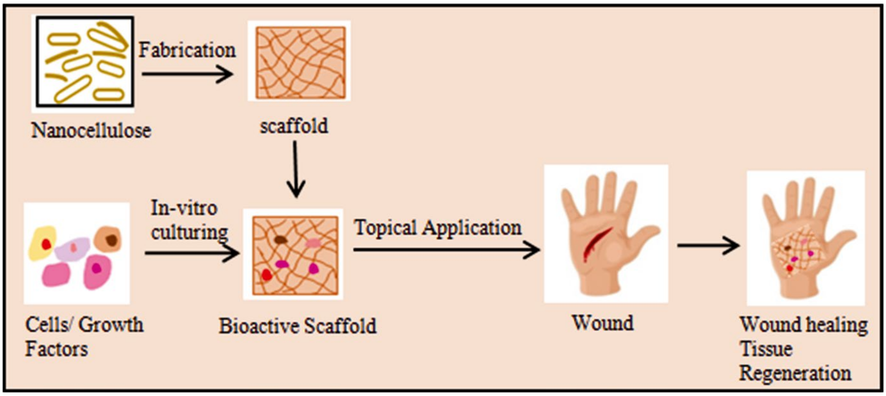

Fig. 4 Illustration of Nanocellulose Scaffolds in Skin Tissue Engineering

proteins, glycosides, polysaccharides, nanoparticles, and local anaesthetics, nanocellulose presents itself as a viable option for accelerating the healing process [58]. They may further give defence against external infections and establish a barrier against bacteria that might cause secondary illnesses. Table 2 depicts some examples of nanocellulose composites for application in skin tissue regeneration. In the following section; we underline different types of nanocellulose and their modification to form nanocomposites for applications in wound healing and skin tissue regeneration.

5.1 Cellulose Nanofibers (CNFs)

In the past years, CNFs have been extensively researched for use in many biomedical domains. CNFs are superior candidates for skin tissue engineering due to their alluring and distinctive qualities, like mechanical strength, porosity, and ultra-light weight (99.98% air by volume), large surface area, strong tensile strength, and low coefficient of heat conductivity. They have similarity to the fibrous structure, size-scale and porosity of extracellular matrix [59]. The CNFs produced from ultrapure wood were demonstrated as promising dressing material for wound management due to their non-toxicity to normal human skin cells even with endotoxin level of 45endotoxin units/g cellulose and relatively high (~7500%) moisture-holding capacity in comparison to commercially available wound dressing (~2500%) [60]. Medical research has proven that moist wound healing ensures fast healing and lowers the risk of scarring and scabs [61]. By supplying water molecules to the site, moist wound healing maintains the wet wound environment, preventing tissue drying and cell death [62].

CNFs have been added as reinforcement material in various biodegradable polymers such aspolylactic acid (PLA), poly (lactic-co-glycolic) acid, polyethylene

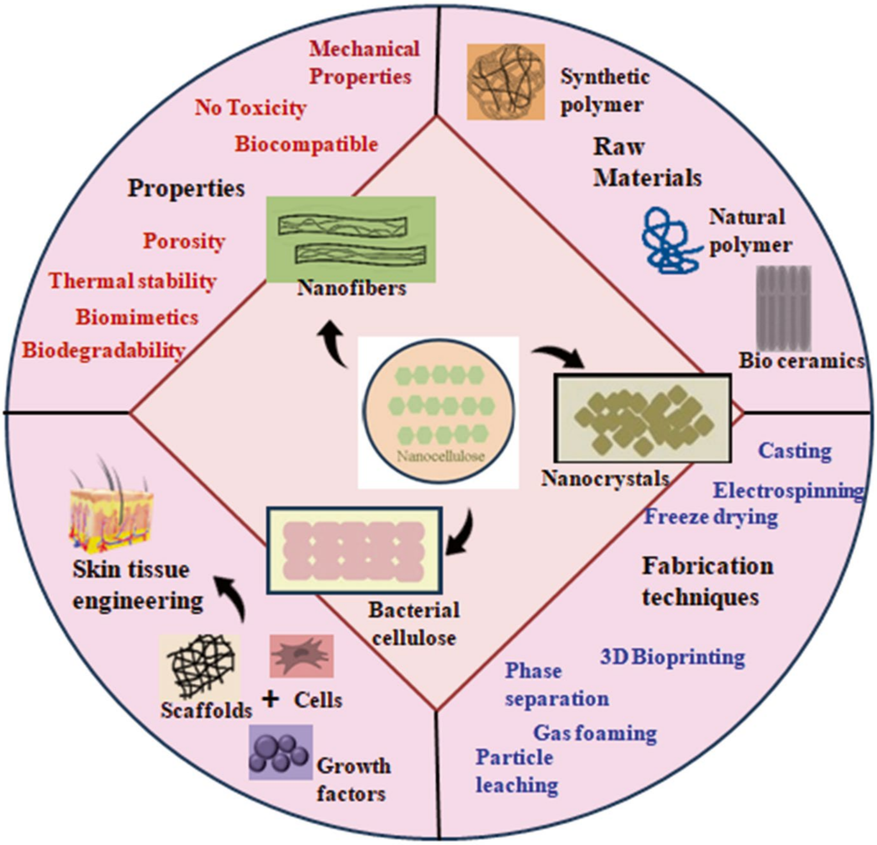
Mechanical Properties
No Toxicity
Biocompatible
Properties
Porosity
Thermal stability
Biomimetics
Biodegradability
Synthetic polymer
Raw Materials
Natural polymer
Bio ceramics
Nanofibers
Nanocellulose
Nanocrystals
Bacterial cellulose
Casting
Electrospinning
Freeze drying
Fabrication techniques
3D Bioprinting
Phase separation
Gas foaming
Particle leaching
Skin tissue engineering
Scaffolds
+ Cells
Growth factors

Fig. 5 Illustration of nanocellulose and its derivatives, modifications, printing methods, and application in skin tissue engineering

glycol, poly(vinyl alcohol) (PVA), poly(globalide) (PGl), etc. Such CNFs-reinforced scaffolds have been shown to be non-toxic, exhibit good cell-migration capacity with enhanced wound closure ability [63–65].

There are studies where CNFs have been modified to alter their physical and chemical characteristics for skin tissue engineering. For instance, 2, 3-epoxy propyl trimethyl ammonium chloride (EPTMAC) or TEMPO-mediated oxidation have been used to modify CNFs in order to introduce a positive or a negative electrical charge [66, 67]. The polyethyleneimine (PEI) crosslinked CNFs and cetyl trimethyl ammonium bromide (CTAB) modified CNFs were demonstrated to be non-toxic to fibroblast cells [68]. CNFs have also been chemically altered into cellulose acetate or hydroxyl ethyl cellulose for skin tissue engineering applications [7]. In one report, dialdehyde nanocellulose fibrils and collagen were crosslinked together through Schiff base to form composite aerogels that demonstrated high-porosities ranged from 90% to 95%, low-densities between 0.02 and 0.03 g cm^{-3}, and strong

Table 2 Nanocellulose composites for application in skin tissue regeneration

Types of Scaffold/ membrane	Cells	Methods	Result	References
Cellulose nanofibers/ poly (vinyl) alcohol	Fibroblast cells	Freeze drying Technique	Biocompatible Material for skin repair	[93]
Cellulose nanocrystals/ PLGA nanofiber membrane	3T3 fibroblast cells	Electro spinning	Better cytocompatibility, fibroblast adhesion, spreading, and proliferation	[75]
Cellulose nanocrystals and alginate	Fibroblast and hepatoma cells	Bioink method	3D bioprinting	[94]
Cellulose nanocrystal film and collagen	3T3 fibroblast	Mixing	No negative effect on the cell morphology, viability, and proliferation and possess good biocompatibility	[95]
Nanocellulose-PEG	–	TEMPO oxidation and Mixing	Higher skin conformability	[96]
Cellulose membrane with chitosan-coated nanofibre	Fibroblast, keratinocyte	Electro spinning	Potential wound dressing	[97]
Gelatin, elastin, Cellulose nanocrystals Scaffolds	Human Dermal Fibroblasts (HDF)	Chemical method	Scaffolds expressed collagen type 1 and α-SMA proteins showing biocompatible with HDF	[98]
Cinnamon infused microfibrillated cellulose/ carboxylated cellulose nanocrystals sponge	Human keratinocytes cells (HaCaT cells), 3T3 L1 cell line from mousa and human umbilical vein endothelial cells (HUVECs) and in-vivo studies.	Mechanical method	Sponges accelerate the blood clotting process and improves cell adherence and proliferation.	[99]

(continued)

Table 2 (continued)

Types of Scaffold/ membrane	Cells	Methods	Result	References
Poly(globalide) (PGl) and regenerated cellulose nanofibers (rCNFs) films	Keratinocytes	Acid hydrolysis, enzymatic ring-opening polymerization	Scaffold for the growing of keratinocyte cells, exhibiting cell metabolic activity	[100]
Cellulose-nanofibrils (CNFs) enriched gelatine (GEL) scaffolds	Mesenchymal Stem Cells (MSCs)	Chemical method and stirring	Extensive Ca^{2+} deposited and can be promising candidates for bone tissue engineering	[101]
Alginate-gelatin-cellulose nanocrystals scaffolds	Human bone marrow-derived mesenchymal stem	3D bioprinting	Structures using CNC exhibit increased mineralization effectiveness and cell proliferation.	[102]
Gelatin-bioactive glass -CNC	L929- Fibroblast	Freeze casting	In comparison to the control, CNC shown a desirable compressive strength, improved wettability, which promotes better cell growth and proliferation, as well as porosity, pore connectivity, and biocompatibility.	[103]
Alginate-nanocellulose Gels	L929- Fibroblasts	Freeze casting	ECM biomimetic construction with favorable mechanical, bio adhesion, growth and cytocompatibility characteristics	[104]
Poly (butylene succinate) (PBS)/ cellulose nanocrystals (CNC) Scaffold	3T3 Fibroblasts	Electrospinning	Scaffold shows biocompatibility and cell viability.	[76]
Cellulose nanofiber, Tempo-oxidated Cellulose nanofiber, Acetylated CNF	H9c2 Cardio-myoblast	3D Printing	High biocompatibility induction of cell attachment, proliferation in cardio-myoblasts in 21 days.	[105]

(continued)

Table 2 (continued)

Types of Scaffold/ membrane	Cells	Methods	Result	References
Cellulose nanofibril-alginate, lignin particles	HepG2 Hepatocellular carcinoma cell line	3D Printing	High swelling ratios in the scaffolds suggested that they had superior water absorption and retention abilities. Additionally, theyexhibit high cell compatibility.	[106]
Polyurethane (PU), cellulose nanofibers	NIH 3T3 mouse skin fibroblasts and human fibroblasts	3D Printing	High swelling ratios in the scaffolds suggested that they had superior water absorption and retention abilities. Additionally exhibit high cell compatibility.	[107]
Cellulose nanofiber-alginate construct	L929 fibroblast	3D Printing	Scaffold show efficient biocompatibility and can be used as wound dressings.	[108]
1,4-butanediol diglycidyl ether crosslinked Tempo- oxidated cellulose nanofibres	Human dermal fibroblasts	3D Printing	Scaffold improves cell proliferation with high rigidity. Mechanical strength in the range of 3–8 kPa	[109]
TEMPO-oxidized CNF, gelatin methacrylate (GelMA)	3T3 fibroblast	3D Printing	Scaffold revealed non-cytotoxicity and biocompatible, proliferation of fibroblast.	[110]
Cellulose nanofibers/ organosilanes and chitosan	Fibroblasts	Freeze casting	Results show effective hemostasis with around 50% reduced blood loss	[111]
TEMPO-oxidized cellulose nanofibres	U937 cells	Freeze casting	*in -vivo* test revealed that CNF scaffolds had a less significant impact on cytokine production than gelatin.	[112]
TEMPO-oxidised Cellulose nanofibres-polyethylene glycol - ZnO	Rabbit ear arterial bleeding model	Freeze drying	Efficient and novel candidate as hemostatic agent.Also shown efficient Antibacterial property.	[113]

(continued)

Table 2 (continued)

Types of Scaffold/membrane	Cells	Methods	Result	References
Polydopamine on electrospun poly(lactic acid)/cellulose nanofibrils	Human MSCs	Electrospinning	Promote cell adhesion, proliferation, and growth of Mesenchymal stem cells.	[114]
Gelatin/diosgenin-nanocellulose hydrogels	Human dermal fibroblast	Freeze casting	Biocompatible, stron potential for wound healing.	[115]

water absorption up to 4000%, in addition to being biocompatible, supporting growth of cells, making them suitable for biological wound dressings and tissue engineering scaffolds [69]. In another very recent study, CNFs bioconjugated with epsilon-poly-L-lysine were used as bio-ink for fabrication of multifunctional 3D composite scaffold with antibacterial efficacy against wound-infecting pathogens and biocompatibility, capable of promoting cell attachment and proliferation essential for enhancing wound healing. The mesoporous structure (2–50 nm) and high specific surface area (232.25m^2/g) of this 3D composite scaffold enabled a high adsorption capacity of red blood cells in wound healing [70]. Similarly, 3D-printed polycaprolactone (PCL) scaffolds coated with wood derived hydrophilic CNFs exhibited improved mesenchymal stem cell adhesion, proliferation, and osteogenic differentiation [71]. CNF hydrogels surface modified with ECM protein, fibronectin, improved attachment ofC3H10T1/2 fibroblast cells [59]. Although these examples demonstrate great potential of CNF based composite scaffolds for the skin tissue engineering, there are however, limited *in vivo* studies.

5.2 Cellulose Nanocrystals (CNCs)

CNCs are increasingly gaining attention due to their outstanding rigidity, low density and high elastic modulus. CNCs are hydrophilic due to abundance of hydroxyl groups (can be modified) making them suitable for use in tissue engineering [72]. The CNCs containing nanocomposites have been implicated in wound healing, CNCs act as reinforcing filler improving the structural and mechanical features of scaffolds used in skin tissue engineering. For instance, incorporation of CNCs into alginate/gelatin scaffold led to enhancement in mechanical strength (from 18 MPa to 45 MPa), desired porous network and moderate swelling behaviour of the scaffold leading to enhanced skin wound healing process with greater collagen deposition, rapid re-epithelialization and faster extracellular matrix remodelling in *in- vivo* skin regeneration [73]. Similarly, addition of CNCs improved the mechanical properties of maleic anhydride (MAH) grafted poly(lactic acid) (PLA) nanocomposite for bone tissue engineering [74]. PLGA/CNC composite nanofiber membranes prepared by electrospinning demonstrated best mechanical properties (similar to those of human skin) at 7 wt.% CNCs, the developed nanocomposite facilitated fibroblast adhesion, spreading, and proliferation making them potential scaffolds for skin tissue engineering applications [75]. Other studies have also reported successful enhancement in mechanical properties of scaffolds by incorporation of CNCs [76, 77].

Uniaxially aligned CNFs incorporated with well oriented cellulose CNCs (20%) demonstrated improved tensile strength and elastic modulus in the fiber alignment direction by 101.7 and 171.6%, respectively in addition to enhanced thermal stability. Furthermore, these scaffolds were non-cytotoxic to human fibroblasts, and human dental follicle cells proliferated rapidly not only on the surface of developed scaffolds but also deep inside the scaffold [78]. CNCs have also been used in the acceleration of wound healing. A nanocomposite of chitosan and kenaf CNCs was developed to stabilize platelet lysate and release it at wound site in a controlled manner for prolonged action. The synthesized hydrogel composites also augmented fibroblast proliferation *in vitro* and fastened wound closures over 72 h [79].

5.3 Bacterial Cellulose (BC)

BC is extensively used in wound healing dressings and skin tissue regeneration due to its biocompatibility, high tensile strength, flexibility, gas and liquid permeability and water absorption capacity. It maintains a suitable moist wound healing environment without excessive dehydration. It was found that BC/chitosan nanocomposites had a superior effect on wound healing with faster epithelial layer formation and better regeneration [80]. Several 3D porous scaffolds with interconnected pore networks have been created using BNC/BC as a sacrificial template. For instance, (BNC)–grafted poly(acrylic acid) (AA)–graphene oxide (BNC/P(AA)/GO) hydrogels and BC/ keratin Nanocomposites have been demonstrated as biocompatible wound dressing materials for accelerated wound healing [81, 82]. Gelatin microspheres were used as porogen and surface modification agent for fabrication of 3D microporous regenerated bacterial cellulose (rBC)/ gelatin scaffolds which displayed higher wound closure efficiency (93%) than pure BC (63%) in experimental mice [83].

Tissue engineers can precisely control the regeneration process through the strategic integration or topical application of particular growth factors within the scaffold material. Figure 6 demonstrates mimetic nanocellulose as skin substitute. The manufactured skin not only replicates the structural integrity of the natural skin but also encourages rapid wound healing with minimal scarring due to the synergistic interaction between growth factors. In one such study, lysozyme, an enzyme frequently present at infected skin wounds, caused the release of epidermal growth factor (EGF) incorporated in BC membranes that sped up the re-epithelialization process and wound repair [84]. Similarly, membrane made of BC and gellan gum

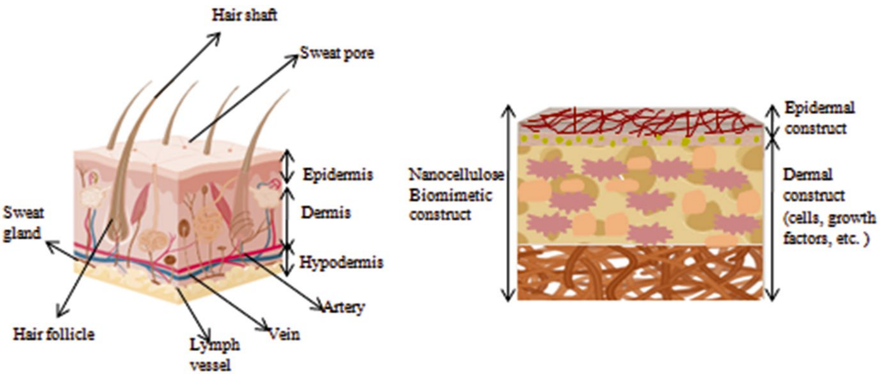

Fig. 6 Biomimetic nanocellulose as skin substitute

with antifungal medication fluconazole was used for delivery of human adipose-derived mesenchymal stem cells (ASCs) at wound site. This ASCs seeded dressing exhibited enhanced healing of second-degree burnsin mice as compared to control (treatment without membrane) [85]. In another report, scaffolds made of BC and keratin hydrogel seeded with adipose stem cells fastened the healing process of burnt wounds in the dorsal region of rabbits and promoted reconstruction of the damaged structure making it a promising dressing in skin tissue regeneration [86].

One of the major concerns while a wound is healing is development of bacterial infection at wound site. In this context, antibacterial properties of BNC may reduce bacterial contamination and encourage development of new cells for enhanced wound closure [87]. An example of such composite is BC combined with modified montmorillonites (Cu-MMT, Na-MMT and Ca-MMT) which have antimicrobial activity. The nanocomposite was demonstrated to have wound healing activity with tissue regeneration, healthy granulation, re-epithelialization and vascularization, making it suitable as artificial skin substitute for burn patients [88]. Small molecules of antibacterial agents and/or accelerative wound healing agents have been impregnated in the pores of BNC which are released in controlled manner at wound site for longer duration. A novel BC wound dressing containing poly hexamethylene biguanide as an antimicrobial agent, and sericin as an accelerative wound healing agent was found to have low inflammatory response when implanted subcutaneously in rats [89]. The clinical trial patch test on the normal skin of healthy volunteers did not show any irritation on the skin, demonstrating dressing's safety. Also, treatment of full-thickness wounds in rats led to reduction in wound size and higher extent of collagen demonstrating its clinical efficacy in wound closure and collagen formation.

Recent research has focussed on the development of strategies for early diagnosis of infections at wound site. A transparent wound covering made of BNC was fabricated that allowed visual real-time monitoring of wound healing using multiphoton laser tomography, optical coherence tomography and confocal laser scanning microscopy [90]. Monitoring wound pH helps in the assessment of wound status and detection of early-stage infections [91]. In one report, pH-responsive dye (bromothymol blue) loaded-mesoporous silica nanoparticles (MSN) were incorporated into BNC to create hydrogel-based wound dressings for continuous monitoring of pH changes at wound site with great spatiotemporal resolution. These pH-responsive BC-based nanocomposites showed excellent wound dressing qualities, such as flexibility, mechanical strength, and water vapor transmission rate [92]. Additionally, these nanocomposites can be used to develop newer wound-care materials such as incorporating bioactive compounds for treatment of difficult wounds in these functional BC-MSN nanocomposites for release at wound site.

6 Commercial Nanocellulose Scaffolds

There are some commercial cellulose dressings like oxidized non regenerated cellulose (Traumastem, Bioster, a.s. Czech Republic) and oxidized regenerated cellulose (Surgicel Original, Ethicon, Inc., New Jersey. USA) available in the market. They are sterilized fibrous, resorbable hemostatic medical devices made from pure, natural cotton using controlled oxidation technology. The effect of oxidized cellulose on fibroblast cells has revealed that oxidized cellulose stimulates cell growth directly, considerably enhancing their metabolic activity. When oxidized cellulose comes in touch with blood, it turns into sticky gel that prevents platelets from being washed out of the wound, allowing the bleeding to cease faster [116]. A study demonstrated that BC membrane (Nanoskin®, Innovatecs Biotechnological Products Ltda, ME, So Carlos-SP, Brazil) promoted healing of full-thickness skin wounds in dorsal region (2 × 4 cm) of guinea pigs to the same extent as control autologous skin implants [117].

Table 3 enlists various commercially available nanocellulose scaffolds for wound healing and skin tissue engineering. Nanocellulose-based dressings require increased attention because of their high biocompatibility, biodegradability, and low cost. Experimental strategies for generating more functional wound dressings, such as adding antibacterial properties and cell growth agents for the healing process, should be prioritized.

7 Challenges

The *in vivo* degradability of nanocellulose is an important concern for its biological use *in vivo*. It must degrade at a controlled rate for specific tissue engineering applications. Due to lack of cellulase enzymes in the human body, the primary component of nanocellulose is deemed non-biodegradable *in-vivo* or, at best, slowly degradable. Nanocellulose-based scaffolds have been demonstrated to degrade *in vitro* and *in vivo*, however, extra pre-treatment and modification, such as oxidation [127] is needed prior its use. In a study, biodegradable cellulase/BC material developed by a simple absorption method was shown to gradually decompose in the

Table 3 Commercially available cellulose scaffolds for wound healing and skin tissue engineering

Commercially available nanocellulose scaffolds	Source	Application	References
FibDex®	Wood based NC	Provide mechanical protection, and enhance wound healing rate.	[118, 119]
Bio Fill®	Bacterial Nanocellulose film	Pain relief, reduced infection, promote healing	[120]
Derma Fill™	Bacterial Nanocellulose	Fluid balance, mechanical matrix which bridge wound bed, promotes granulation and re-epithelialization and antimicrobial.	[121]
X-cell®	Microbial Nanocellulose	Provide hydration, maintain optimal healing environment, reduction in wound size, and increases granulation tissue and re-epithelialization.	[122]
Bioprocess®	Bacterial Nanocellulose	Optimal wound healing environment	[123]
Bionext®	Bacterial Nanocellulose	Promote cell metabolism, antimicrobial, reduces pain, decrease electrolyte loss and reconstruct tissue	[124]
Membracel®	Bacterial Nanocellulose	Temporary skin substitute, enhance rate of skin regeneration.	[125]
Epicite®	Bacterial Nanocellulose	Absorbs wound exudates, low pain, wound healing, provide moist environment.	[126]

simulated body fluid after 24 weeks, and the degradation rate could be modified by altering the cellulase level, indicating the cellulase/BC material to be biocompatible both *in- vitro* and *in -vivo* [128]. In another report, cellulose scaffolds were oxidised with periodate and it was found that oxidized scaffolds lost weight in the cell culture medium, the cellulose molecular degradation was due to Schiff base formation between the aldehyde and amino groups through a Maillard reaction. It was concluded that the biodegradability of cellulose and inflammation due to scaffolds *in vivo* could be controlled by oxidation of scaffolds [129]. In a recent study, 3D printed CNF/chitosan hydrogel scaffolds' *in vitro/in vivo* breakdown kinetics was managed by encapsulating the enzyme cellulose in chitosan–caseinate nanoparticles which were incorporated in the bioink [130]. In another report, an antibacterial nanocomposite comprising oxidized BC, chitosan, and collagen was found to have higher hemostatic capacity and *in vivo* biodegradability in a rat liver injury model, as compared to commercial oxidized regenerated cellulose (ORC, commonly known as Surgicel gauze) [131]. Further research is required to optimise the use of nanocellulose in biomedical applications while also ensuring its safe and sustainable production.

8 Conclusion

In conclusion, this chapter summarized brief overview of the types of nanocellulose and their syntheses methods with emphasis on their specific properties such as mechanical property, biocompatibility, non-toxicity and surface chemistry. Their use as scaffolds for wound repair and skin tissue engineering have also been discussed. The potential of tissue-engineered skin substitutes raises expectations for future developments in skin regeneration and wound-healing treatments. In the biomedical industry, where there is a significant need for innovative scaffolds, numerous scaffolds have been developed for tissue engineering applications. The efficacy of tissue engineering depends on the materials and production methods utilized to build these scaffolds. In order to establish a favourable environment for mending injured tissues and encouraging the regeneration of new cells, these scaffolds must imitate the extracellular matrix (ECM) and have good biocompatibility. Nanocellulose holds great promise for a variety of uses in industry, technology, biotechnology, and medicine, including tissue engineering and wound healing. It can be used as a scaffold for cell development, to transfer cells into wounds, and as a material for improved wound dressings that incorporate drug delivery, transparency, and sensitivities. So far, nanocellulose has been demonstrated to be useful in repairing injured skin, promoting active wound healing, and regenerating diverse tissues. The non-degradability of nanocellulose in the human body, however, is a barrier to its direct application in skin tissue engineering as a scaffold for skin cells since scaffolds that stay in the skin may result in scarring and other issues. More strategies are needed to ensure degradability of nanocellulose *in vivo*. In summary, nanocellulose holds promise for skin tissue regeneration and wound healing, and its prospective applications in this field are still being investigated.

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Navigating Infectious Challenges: The Nanomedicine Era

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1 Introduction to Nanomedicine

Nanomedicine represents a cutting-edge field at the intersection of nanotechnology and medicine. It offers innovative solutions for preventing, diagnosing, and treating diseases by leveraging nano-sized tools and materials [1]. These advancements hold great promise for improving healthcare outcomes and addressing various medical challenges with increased precision and efficacy. Nanomedicine utilizes nano-sized tools to diagnose, prevent, and treat diseases, while also enhancing our understanding of complex disease mechanisms. Its overarching aim is to elevate the quality of life through targeted therapies, early disease detection, and improved treatment outcomes [2]. By working at the molecular and cellular levels, nanomedicine offers precise interventions, targeted drug delivery, and enhanced imaging techniques, leading to more effective and personalized healthcare [3]. One of the key advantages of nanomedicine is its ability to target specific cells or tissues, minimizing side effects and improving treatment efficacy [4]. Nano-sized drug delivery systems, such as nanoparticles and liposomes, can be designed to release therapeutic agents at precise locations within the body, reducing the dose required and enhancing therapeutic outcomes [5]. Additionally, nanotechnology enables the development of advanced imaging techniques, such as nanoparticle-based contrast agents for

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improved detection and monitoring of diseases like cancer [6]. These capabilities highlight the transformative potential of nanomedicine in revolutionizing healthcare delivery.

2 Nanoparticles and Drug Delivery

Nanoparticles are minute particles typically measuring between 1 and 100 nm in size, making them exceptionally small compared to cells and even viruses [7]. They can be engineered from various materials such as metals, polymers, or lipids, each offering unique properties for biomedical applications [8]. In nanomedicine, nanoparticles play a crucial role in drug delivery by encapsulating therapeutic agents and delivering them to specific targets in the body [9]. This targeted drug delivery approach minimizes systemic exposure, reduces side effects, and enhances the therapeutic efficacy of the drugs [10]. There are several types of nanoparticles used in drug delivery, each with its unique properties and applications are depicted in Fig. 1.

1. ***Metallic Nanoparticles***: These nanoparticles are typically composed of metals such as gold, silver, and iron oxide [11]. They exhibit properties like surface plasmon resonance (in gold and silver nanoparticles) or magnetic properties (in iron oxide nanoparticles), making them valuable for imaging, drug delivery, and therapeutic purposes [12].
2. ***Polymeric Nanoparticles***: These nanoparticles are made from biocompatible polymers such as poly (lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), and chitosan. They are commonly used for drug delivery due to their ability to encapsulate drugs, protect them from degradation, and control their release kinetics [13].
3. ***Lipid-Based Nanoparticles***: Lipid nanoparticles include liposomes, lipid nanocarriers, and solid lipid nanoparticles. They are composed of lipids and phospholipids, mimicking the structure of cell membranes. Lipid-based nanoparticles are efficient drug carriers, especially for delivering hydrophobic drugs, and can be modified for targeted delivery and controlled release [14].
4. ***Carbon-Based Nanoparticles***: Carbon nanoparticles like carbon nanotubes and graphene have unique physicochemical properties, such as high surface area and conductivity. They are explored for various biomedical applications, including drug delivery, biosensing, tissue engineering, and imaging [15].

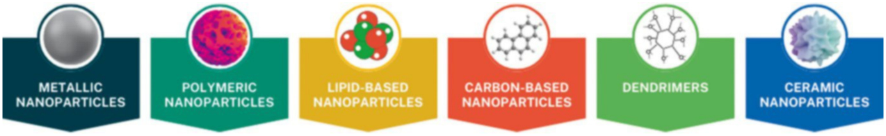

Fig. 1 Types of nanoparticles used in drug delivery

5. ***Dendrimers***: Dendrimers are highly branched synthetic macromolecules with well-defined structures. They can carry drugs in their interior cavities and on their surface, enabling precise control over drug loading and release. Dendrimers are investigated for targeted drug delivery and diagnostic imaging [16].
6. ***Ceramic Nanoparticles***: Ceramic nanoparticles like silica nanoparticles and hydroxyapatite nanoparticles are biocompatible and have tunable properties. They are used for drug delivery, imaging, and regenerative medicine applications due to their stability, porosity, and surface modification capabilities [17].

These diverse types of nanoparticles offer versatile platforms for developing advanced nanomedicine technologies, ranging from targeted drug delivery systems to diagnostic agents and therapeutic interventions. The combination of nanoparticles and drug delivery systems represents a synergistic approach in nanomedicine. By incorporating drugs into nanoparticles, researchers can overcome challenges such as poor solubility, rapid degradation, and limited bioavailability [9]. Moreover, Nanoparticles have the capability to be functionalized with targeting ligands or stimuli-responsive components, providing a means for accurate control of drug release at the intended site or in response to specific biological cues [18]. This combination of nanotechnology and drug delivery holds tremendous potential for developing personalized and effective treatments for a wide range of diseases, paving the way for more efficient and targeted therapies in modern medicine.

3 Diagnostic Nanotechnology

Diagnostic nanotechnology refers to the application of nanoscale materials and devices in the field of medical diagnostics. By leveraging the unique properties of nanoparticles and nanomaterials, nanotechnology enables highly sensitive, rapid, and accurate detection of biomolecules, pathogens, and disease markers. This innovative approach revolutionizes traditional diagnostic methods by offering enhanced sensitivity, specificity, and multiplexing capabilities. From nanoparticle-based biosensors to nanomaterial-enhanced imaging techniques, diagnostic nanotechnology (Fig. 2) holds immense promise for early disease detection, personalized medicine, and improved healthcare outcomes.

3.1 Nanosensors for Pathogen Detection

Nanosensors have emerged as powerful tools for the detection of pathogens due to their high sensitivity, specificity, and rapid response capabilities [19]. Nanosensors are nanoscale devices or structures designed to detect and respond to specific physical, chemical, or biological stimuli [20]. The nanoscale dimensions and large specific surface area of nanosensors, which include functionalized nanoparticles such

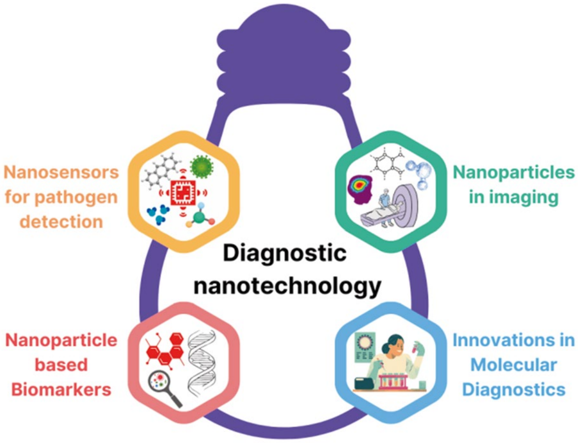
Nanosensors for pathogen detection
Nanoparticles in imaging
Diagnostic nanotechnology
Nanoparticle based Biomarkers
Innovations in Molecular Diagnostics

Fig. 2 Nanotechnology in diagnosis and imaging

as gold nanoparticles, quantum dots, and magnetic nanoparticles, enable highly effective interaction with pathogenic agents at the molecular level [21]. This heightened interaction significantly boosts the sensitivity and selectivity of biosensors, making them invaluable for accurately detecting and measuring target substances or properties [22]. By selectively binding to specific biomolecules or molecular markers associated with pathogens, they trigger measurable signals such as changes in optical, electrical, or magnetic properties, which are then detected and quantified [23]. This targeted approach enables precise and accurate pathogen detection in various applications, from healthcare to food safety and environmental monitoring. Moreover, nanosensors can be integrated into miniaturized and portable devices, allowing for on-site and real-time detection of pathogens, which is crucial for timely intervention and disease control, especially in resource-limited settings or during outbreaks. Additionally, nanosensors offer multiplexing capabilities, enabling simultaneous detection of multiple pathogens or biomarkers in a single assay, thereby enhancing the efficiency and accuracy of pathogen detection for improved disease management and public health surveillance.

There are several types of nanosensors used in pathogen detection, each with unique mechanisms and advantages:

Plasmonic Nanosensors

Plasmonic nanosensors are a type of nanoscale sensor that leverages the distinctive optical characteristics of metallic nanoparticles, including those made of gold and silver, for pathogen detection. These nanoparticles exhibit a phenomenon called surface plasmon resonance (SPR), which is the collective oscillation of electrons on their surface when they are exposed to light of a specific wavelength [24]. In the context of pathogen detection, plasmonic nanoparticles are functionalized with molecules, such as antibodies or DNA probes that can bind specifically to target pathogens, such as bacteria or viruses. When these functionalized nanoparticles come into contact with the target pathogens, the binding event causes a change in the local refractive index and dielectric properties around the nanoparticles. This change in the local environment alters the SPR properties of the nanoparticles, leading to shifts in their absorption or scattering spectra. These spectral changes can be detected using techniques such as UV-Vis spectroscopy or surface plasmon resonance spectroscopy, allowing for the quantitative measurement of the bound pathogens [25]. Plasmonic nanosensors offer several advantages for pathogen detection. Firstly, they are highly sensitive, capable of detecting even low concentrations of pathogens due to the strong signal amplification provided by the SPR effect. Secondly, they are specific, as the functionalization of the nanoparticles with specific recognition molecules ensures selective binding to target pathogens. Additionally, plasmonic nanosensors can be integrated into portable and miniaturized devices, enabling rapid on-site detection without the need for complex laboratory equipment. Table 1 summarizes some of the reported nanosensors based on surface plasmon resonance (SPR) [26].

Fluorescent Nanosensors

Fluorescent nanosensors are a class of nanoscale sensors used in pathogen detection that rely on the fluorescence properties of nanoparticles, such as quantum dots and fluorescent polymers. These nanoparticles are engineered to emit fluorescent signals when they interact with target pathogens, enabling sensitive and specific detection. In the case of quantum dots, which are semiconductor nanoparticles, their unique size-dependent fluorescence properties allow for tunable emission wavelengths. This characteristic makes them versatile as labels or probes for detecting different types of pathogens. Quantum dots can be functionalized with molecules

Table 1 Examples of SPR-based nanosensors

Nanosensor/material	Analyte	Limit of detection	Reference
Gold nanoparticles	Prostate-specific antigen (PSA)	0.2 ng/mL	[27]
Gold/silver nanoparticles	Glucose	3 μM	[28]
Spike-like gold nanoparticles	Hemagglutinin	1 pM	[29]
Gold nanodisk arrays	Prostate-specific antigen (PSA)	1.49 ng/mL	[30]
Gold nanorods	25-hydroxyvitamin D3	0.1 ng/mL	[31]
Gold nanoparticles	Interleukin-6	0.8×10^{-12} M	[32]
Gold 3D nanopopcorn	Influenza A/H1N1 virus	97 PFU/mL	[33]
Gold nanopillars	Procalcitonin	0.5 ng/mL	[34]
Gold nanoparticles	Plasmodium falciparum lactate dehydrogenase	1 pg/mL	[35]
Gold nanoparticles	SARS-CoV-2 viral RNA	0.18 ng/μL	[36]

like antibodies or DNA probes that specifically bind to target pathogens. When the quantum dots bind to the pathogens, they emit fluorescence that can be detected and quantified using fluorescence spectroscopy or imaging techniques. Fluorescent polymers are another type of fluorescent nanosensor used in pathogen detection. These polymers can be designed to exhibit fluorescence in the presence of specific target molecules or pathogens. By functionalizing the polymers with recognition elements, such as aptamers or molecularly imprinted polymers, they can selectively bind to target pathogens, triggering a fluorescence signal that indicates the presence of the pathogens. The advantages of fluorescent nanosensors in pathogen detection include their high sensitivity, as they can detect pathogens at low concentrations, and their specificity, as they can differentiate between different pathogens based on their binding interactions. Additionally, fluorescent nanosensors offer real-time detection capabilities and can be integrated into imaging systems for visualizing the distribution of pathogens in biological samples. Table 2 outlines several nanosensors that have been reported, highlighting those based on fluorescence.

Magnetic Nanosensors

Magnetic nanosensors are advanced nanoscale devices used in pathogen detection, relying on the magnetic properties of nanoparticles, such as iron oxide nanoparticles, for sensitive and specific detection of pathogens. These nanosensors are functionalized with molecules, such as antibodies or aptamers that selectively bind to target pathogens, allowing for precise detection. The functionalized magnetic nanoparticles are dispersed in a sample containing the target pathogens. When the nanoparticles bind to the pathogens, they create magnetic signals that can be

Table 2 Examples of fluorescent based nanosensors

Nanosensor/material	Analyte	Limit of detection	Reference
N-acetylcysteine quantum dots	L. monocytogenes	1×10^3 CFU/mL	[37]
FeO-nanoparticles and quantum dots	E. coli	1×10^2 CFU/mL	[38]
Iron core gold nanoparticles	S. enteritidis	32 Salmonella/mL	[39]
Silica nanoparticles	E. coli	8 CFU/mL	[40]
Silica nanoparticles	AFB1 from filamentous fungi	0.214 pg/mL	[41]
Gold nanoparticles	S. aureus	10^1–10^4 CFU/mL	[42]
Magnetic nanoparticles	S. typhimurium	53 CFU/mL	[43]
Quantum dots	S. typhimurium	43 CFU/mL	[44]
Quantum dots	S. typhimurium and V. parahaemolyticus	10 CFU/mL 10^2 CFU/mL	[45]
Quantum dots nanobeads	S. typhimurium	10^{-1} CFU/mL	[46]

detected using magnetic resonance-based techniques, such as magnetic resonance imaging (MRI) or magnetic sensors. The binding event between the nanoparticles and pathogens induces changes in the magnetic properties, such as relaxation times or magnetic susceptibility, which can be quantified to determine the presence and concentration of the pathogens. One of the key advantages of magnetic nanosensors is their high sensitivity, as they can detect pathogens at very low concentrations due to the amplification of magnetic signals by the nanoparticles. Additionally, magnetic nanosensors offer rapid detection capabilities and can be integrated into portable and miniaturized devices for on-site testing. This makes them valuable in applications where quick and accurate detection of pathogens is essential, such as in clinical diagnostics, point-of-care testing, and environmental monitoring. Furthermore, magnetic nanosensors can be multiplexed to detect multiple pathogens simultaneously in a single assay, enhancing their efficiency and utility in complex sample analysis. They are also compatible with magnetic separation techniques, allowing for the isolation and purification of target pathogens from complex biological samples before detection. Table 3 presents an overview of nanosensors reported in studies, focusing on those utilizing magnetic properties [47].

Electrochemical Nanosensors

Electrochemical nanosensors are cutting-edge devices used in pathogen detection that leverage the electrochemical properties of nanomaterials, such as carbon nanotubes, graphene, or metallic nanoparticles, for sensitive and specific detection of pathogens. These nanosensors function based on the changes in electrical signals or currents generated when target pathogens interact with the nanomaterials. In electrochemical nanosensors, the nanomaterials are functionalized with molecules like antibodies, DNA probes, or enzymes that can bind selectively to target pathogens. When the pathogens bind to the nanomaterials, they induce changes in the electrical conductivity, impedance, or electrochemical reactions of the nanomaterials, resulting in measurable changes in the electrochemical signals. There are several types of electrochemical nanosensors used in pathogen detection, including amperometric,

Table 3 Examples of nanosensors based on magnetic properties

Nanosensor/material	Analyte	Limit of detection	Reference
Magnetic nanoparticles	Mycobacterium tuberculosis	20 CFU/mL	[48]
Mn-doped iron oxide nanoparticles	Cancer cells	2 cells in 1 μL	[49]
Iron oxide NP	Antibody	<1 nM	[50]
Iron oxide nanoparticles	Bacteria	1.1×10^5 bacteria/20 μL	[51]
Cubic FeCo nanoparticles	Protein	2×10^6 proteins/2 μL	[52]
Antiferromagnetic nanoparticles	DNA	10 pM	[53]
Iron oxide nanoparticles	Protein	2.4 pM	[54]

potentiometric, and impedimetric sensors. Amperometric sensors measure the current produced by electrochemical reactions when target pathogens bind to the nanomaterials, while potentiometric sensors measure changes in electrical potential. Impedimetric sensors, on the other hand, detect changes in impedance caused by the binding events between the nanomaterials and pathogens. The advantages of electrochemical nanosensors in pathogen detection include their high sensitivity, rapid response time, and potential for miniaturization and integration into portable devices. They can detect pathogens at low concentrations and offer real-time monitoring capabilities, making them suitable for point-of-care diagnostics and environmental monitoring applications. Furthermore, electrochemical nanosensors can be multiplexed to detect multiple pathogens simultaneously in a single assay, enhancing their versatility and utility in complex sample analysis. They are also cost-effective and compatible with automated systems, allowing for high-throughput screening of samples. Table 4 provides an overview of reported nanosensors that rely on changes in electrochemical properties.

Surface-Enhanced Raman Scattering (SERS) Nanosensors

Surface-Enhanced Raman Scattering (SERS) nanosensors are highly sensitive and specific devices used in pathogen detection that rely on the surface-enhanced Raman scattering effect exhibited by metallic nanoparticles, typically gold or silver nanoparticles [63]. These nanosensors enhance the Raman signals of molecules near their surfaces, enabling the detection and identification of pathogens based on their unique Raman spectra [64]. In SERS nanosensors, the metallic nanoparticles are functionalized with molecules, such as Raman-active molecules or antibodies that can bind selectively to target pathogens [65]. When the target pathogens bind to the nanoparticles, they induce a significant enhancement in the Raman scattering signals of nearby molecules, resulting in strong and distinctive Raman spectra that can be detected and analyzed. The key advantage of SERS nanosensors in pathogen detection is their exceptional sensitivity, capable of detecting pathogens at very low concentrations due to the intense signal enhancement provided by the nanoparticles. This high sensitivity allows for the detection of trace amounts of pathogens in

Table 4 Examples of electrochemical based nanosensors

Nanosensor/material	Analyte	Limit of detection	Reference
Silver nanoparticles	E. coli	150 CFU/mL	[55]
Silver nanoparticles	S. aureus	1.0 CFU/mL	[56]
Silver nanoparticles	S. aureus	1.0 CFU/mL	[57]
Chitosan-silver NPs	G-lipopolysaccharide	248 CFU/mL	[58]
Silver nanoparticles	E. coli	2 CFU/mL	[59]
Silica nanoparticles	E. coli	10^3 CFU/mL	[60]
Multiwalled carbon nanotubes	E. coli	0.8 CFU/mL	[61]
Iron oxide encapsulated quantum dots	Norovirus	69 RNA copies/mL	[62]

complex samples, such as biological fluids or environmental samples. Additionally, SERS nanosensors offer high specificity, as they can differentiate between different pathogens based on their unique Raman spectra. This specificity is crucial for accurate identification and discrimination of target pathogens from background noise or interfering substances in the sample. SERS nanosensors are also versatile and can be integrated into miniaturized and portable devices for on-site testing and real-time monitoring. They are compatible with multiplexing techniques, enabling simultaneous detection of multiple pathogens in a single assay, thereby enhancing efficiency and throughput. Table 5 outlines several nanosensors reported in literature, specifically focusing on those that utilize Surface-Enhanced Raman Scattering (SERS).

These different types of nanosensors in pathogen detection offer a range of options for sensitive, specific, and rapid detection of various pathogens and other analytes making them invaluable tools in healthcare, biodefense, food safety, and environmental monitoring.

3.2 *Nanoparticles in Imaging*

The ongoing imperative for early disease detection and diagnosis drives continuous advancements in imaging modalities and contrast agents. Nanoparticles have revolutionized medical imaging, offering unique properties that enhance diagnostic accuracy and therapeutic precision. Ranging from 1 to 100 nm, these tiny particles boast a high surface area-to-volume ratio and tunable surface chemistry, making them ideal for applications such as contrast enhancement, targeted drug delivery, and molecular imaging. In magnetic resonance imaging (MRI), nanoparticles like superparamagnetic iron oxide nanoparticles (SPIONs) and gadolinium-based nanoparticles serve as contrast agents, providing detailed insights into tissue structure and function. Similarly, in computed tomography (CT), nanoparticles made of materials like gold or bismuth act as high-density contrast agents, improving visualization of anatomical structures and abnormalities. In positron emission tomography (PET) and single photon emission computed tomography (SPECT), radioactive nanoparticles serve as tracers to track biological processes and detect diseases at the

Table 5 Examples of Surface-Enhanced Raman Scattering based nanosensors

Nanosensor/material	Analyte	Limit of detection	Reference
Metal complex-ligated gold nanoparticles	S. sonnei	10^3 cfu/mL	[66]
2-mercaptoisonicotinic acid functionalized gold nanoparticles	Hg^{2+}	1.0×10^{-7} M	[67]
Aptameric biosensor	Cocaine	1 μm	[68]
Gold-nanoneedles array	SARS-CoV-2	80 copies/mL	[69]
Microneedle array	Glucose	20 mM	[70]
Au@Ag core-shell nanorod	E. coli	10^2 CFU/mL	[71]

molecular level with high sensitivity. Nanoparticles also play a crucial role in optical imaging techniques, providing bright fluorescence or photoacoustic signals for precise cellular and molecular imaging. Despite challenges such as toxicity and regulatory approvals, ongoing research aims to develop biocompatible nanoparticles for personalized medicine, targeted therapies, and advanced imaging modalities, heralding a new era in medical diagnostics and treatments [72].

Nanoparticles in MRI Imaging Applications

Magnetic resonance imaging (MRI) is a robust imaging technique that has been extensively utilized in clinical settings for a considerable period. It relies on the spin of protons within a magnetic field when stimulated by a radio frequency pulse [73]. By detecting the nuclear magnetic resonance signals emitted by protons in the human body, MRI delivers exceptional spatial and temporal resolutions along with remarkable inherent soft-tissue contrast [74]. In MRI, nanoparticles like superparamagnetic iron oxide nanoparticles (SPIONs) and gadolinium-based nanoparticles serve as contrast agents by altering the relaxation times of surrounding water molecules, leading to enhanced signal intensity and improved image resolution. Table 6 summarizes examples of MRI contrast nanoparticles from the literature, detailing their nanoparticle composition and biomedical applications.

Nanoparticles in CT Imaging Applications

Computed tomography (CT) is a medical imaging technique that utilizes X-rays and computer processing to produce detailed cross-sectional images of the body [82]. The contrast agent used for CT scans is typically iodine-based, such as iodinated contrast media (ICM) [83]. These agents contain iodine, which has a high atomic number and effectively absorbs X-rays, enhancing the contrast between different tissues and structures in the body. This contrast enhancement allows for better

Table 6 Examples of MRI contrast nanoparticles and biomedical applications

Nanoparticles/composition	Biomedical application	Reference
Alpha(nu)beta(3)-Gd	Imaging angiogenesis	[75]
Liposomal gadolinium	Imaging placenta as blood-pool contrast	[76]
RBC encapsulated iron particles	Blood-pool contrast with longer life-time	[77]
PEGMnCaP nanoparticles	pH-activatable contrast in cancer	[78]
Mn-nanotexaphyrin	Imaging lymph node	[79]
FibPep-ION-micelles	Detecting and imaging thrombus	[80]
Mn-SPIO micella	High power liver imaging contrast	[81]
HBPFPE-aptamer	Detecting breast cancer	[81]

visualization of blood vessels, organs, tumors, and abnormalities during CT imaging. However, traditional iodinated contrast agents have limitations, including a short circulation time in the bloodstream and a risk of adverse reactions such as allergic reactions and kidney damage, particularly in patients with pre-existing renal impairment. Nano-contrast agents are being explored as alternatives to traditional iodinated contrast agents in CT imaging. These nanoparticles, often made of materials like gold or bismuth, offer several advantages over conventional contrast agents. Firstly, they have a longer circulation time in the bloodstream due to their smaller size and can be functionalized with targeting ligands to enhance specificity for certain tissues or cells. Secondly, nano-contrast agents exhibit high X-ray attenuation properties, providing strong contrast enhancement in CT images. Thirdly, they have shown reduced toxicity compared to iodinated contrast agents, making them potentially safer for use in patients with renal impairment or allergies [84]. Table 7 summarizes examples of nano-imaging agents for CT from the literature, detailing their composition and applications in medical imaging.

Nanoparticles in PET Imaging Applications

Positron emission tomography (PET) stands as a robust and extensively applied nuclear medicine technology characterized by deep tissue penetration, exceptional sensitivity, and real-time quantitative imaging analysis [92]. Positron emission tomography (PET) is a medical imaging technique that uses radioactive tracers to detect and visualize physiological processes and metabolic activity within the body. It involves the injection of a radioactive substance, known as a radiotracer, which emits positrons. These positrons interact with electrons in the body, producing gamma rays that are detected by a PET scanner [93]. By mapping the distribution and concentration of the radiotracer, PET scans can provide valuable information about organ function, tissue viability, and the presence of diseases such as cancer, cardiovascular disorders, and neurological conditions. In PET imaging, radioactive nanoparticles like radiolabeled liposomes or dendrimers are used as tracers to track molecular processes in vivo. These nanoparticles emit positrons that are detected by

Table 7 Nano-imaging agents for CT and applications in medical imaging

Nanoparticles/composition	Biomedical application	Reference
PSMA-aptamer conjugated AuNP	Imaging prostate cancer cells	[85]
Liposomal iodine	Imaging macrophage-rich atherosclerotic plaques	[86]
Gold nanoparticles	Incorporating RBC to image blood flow	[87]
Gold nanoparticles	Labelling tumor cells to image tumor growth	[88]
Liposomal iodine	Imaging tumor	[89]
Gold nanoparticles	Tracking mesenchymal stem cells	[90]
Tantalum oxide	Producing greater imaging capability than iodine	[91]

PET scanners, enabling quantitative assessment of physiological processes and molecular interactions within the body. Table 8 summarizes examples of nanoparticles utilized in PET/SPECT imaging as reported in the literature, detailing their applications and characteristics.

Nanoparticles in Fluorescence Imaging Applications

Fluorescence imaging is a powerful tool in biomedical research and diagnostics, offering high sensitivity and specificity for visualizing biological processes at the cellular and molecular levels [101]. It enables real-time observation of dynamic events, multiplexing capabilities for studying multiple targets simultaneously, and personalized approaches for disease diagnosis and treatment. However, challenges such as limited tissue penetration depth, autofluorescence interference, photobleaching, and background noise require careful consideration and ongoing advancements in imaging technologies and probe design to maximize the potential of fluorescence imaging in understanding complex biological systems and improving patient outcomes.

Nanoparticles have significantly advanced fluorescence imaging techniques by offering unique properties that enhance sensitivity, specificity, and versatility in biomedical applications. Fluorescent nanoparticles like quantum dots (QDs) and organic dye nanoparticles which emit intense fluorescence signals and can be tailored to target specific biomolecules or cellular structures [102]. Additionally, nanoparticles offer benefits such as photostability, tunable emission wavelengths, and the ability to carry multiple imaging agents or therapeutic payloads simultaneously. Their small size allows for efficient cellular uptake and distribution, enabling detailed and precise imaging of biological samples. Moreover, nanoparticles in fluorescence imaging contribute to advancements in areas such as live cell imaging, disease diagnosis, drug delivery monitoring, and biomarker detection. Ongoing

Table 8 Nanoparticles utilized in PET/SPECT imaging and applications

Nanoparticles/composition	Biomedical application	Reference
^{18}F-labeled DBCO-PEGylated MSN	Imaging tumor	[94]
$^{125/124}$I-labeled anti-ICAM-1/PVPh-NPs	Detecting pulmonary inflammation	[95]
^{64}Cu labeled CANF-comb nanoparticle	Imaging natriuretic peptide clearance receptor in prostate cancer	[96]
^{64}Cu-TNP	Imaging macrophages in inflammatory atherosclerosis	[97]
^{64}Cu labeled CANF-comb nanoparticle	Imaging atherosclerosis in artery	[98]
^{125}I silver nanoparticle	Monitoring distribution of nanoparticles	[99]
^{111}In labeled lipid/calcium/phosphate NPs	Imaging lymph node metastasis	[100]

research continues to explore novel nanoparticle designs, functionalization's, and imaging strategies to further improve the sensitivity, resolution, and multiplexing capabilities of fluorescence imaging in biomedical research and clinical applications. Table 9 outlines nanoparticles used in fluorescence imaging, detailing their applications and features as per literature sources.

Overall, nanoparticles in imaging represent a burgeoning field with immense potential for improving diagnostic accuracy, monitoring disease progression, and guiding targeted therapies. Their versatility, biocompatibility, and ability to be tailored for specific imaging modalities make them indispensable tools in the realm of medical imaging, promising advancements in precision medicine and personalized healthcare.

3.3 Nanoparticle-Based Biomarkers

Nanoparticle-based biomarkers represent a cutting-edge approach in the diagnosis, prognosis, and monitoring of infectious diseases, heralding a new era in precision medicine [110]. These innovative biomarkers harness the unique properties of nanoparticles to enable highly sensitive and specific detection of disease-associated molecules, providing valuable insights into disease progression and treatment response. In the context of infectious diseases, nanoparticle-based biomarkers offer several distinct advantages, including enhanced sensitivity, multiplexing capabilities, and the ability to detect low-abundance biomolecules [111]. An essential benefit of nanoparticle-based biomarkers is their capability to surmount the limitations of traditional biomarker assays, such as enzyme-linked immunosorbent assays (ELISA), which may lack the sensitivity required for early disease detection or fail to distinguish between closely related biomarkers [112]. Nanoparticle-based biomarkers can be designed to target various types of infectious agents, including bacteria, viruses, fungi, and parasites. For instance, gold nanoparticles functionalized with specific antibodies or aptamers can selectively bind to surface antigens or nucleic acids of pathogens, enabling their rapid detection in clinical samples [113].

Table 9 Nanoparticles used in fluorescence imaging and their applications

Nanoparticles/composition	Biomedical application	Reference
Cy5.5-substrate/AuNP	Detecting protease activity	[103]
CNP(Mtx-Asp-FITC)	Monitoring therapeutic drug delivery	[104]
Cy7.5-INCeRT	Monitoring drug diffusion	[105]
Au@IR-pHPMA	Detecting lymph node	[106]
Perylenediimide-containing polysiloxane core and silica shell	Detecting nanotoxicity in alive cells	[107]
Cationic oligofluorene substrated POSS	Imaging double-stranded DNA	[108]
PFVBT(AuNP-PLGA)/(PLGA-PEG$_{2000}$-folate)	Detecting MCF-7 cell in breast cancer	[109]

Similarly, magnetic nanoparticles coated with molecular probes can be used for the sensitive detection of viral RNA or DNA in blood or respiratory specimens, aiding in the diagnosis of viral infections such as influenza or COVID-19 [114]. In addition to pathogen-specific biomarkers, nanoparticle-based platforms can also be employed to detect host biomarkers indicative of immune responses or disease severity [115]. For example, cytokines, chemokines, and other immune mediators released during infection can be quantified using nanoparticle-based immunoassays, providing valuable information about the host's immune status and the progression of infectious diseases. Moreover, the development of multiplexed nanoparticle-based assays allows for the simultaneous detection of multiple biomarkers within a single sample, offering a comprehensive view of the host-pathogen interaction and disease dynamics [116]. Furthermore, nanoparticle-based biomarkers hold promise for monitoring treatment responses and guiding therapeutic decisions in infectious diseases. Clinicians can evaluate the effectiveness of antimicrobial treatments by analyzing fluctuations in biomarker levels across time, monitor the development of drug resistance, and identify patients at risk of treatment failure or disease recurrence [117]. Moreover, the use of point-of-care nanoparticle-based assays enables rapid and on-site diagnosis of infectious diseases, facilitating timely interventions and improving patient outcomes, particularly in resource-limited settings or during outbreaks. Here are some detailed examples of nanoparticle-based biomarkers in infectious disease diagnosis and monitoring:

Gold Nanoparticle-Based Assays for Viral Detection

Gold nanoparticles have been extensively used in the development of rapid diagnostic assays for viral infections. For instance, researchers have developed lateral flow assays (LFAs) using gold nanoparticles functionalized with viral-specific antibodies or aptamers for the detection of viruses such as HIV, influenza, and Zika [118]. In these assays, the presence of viral antigens in clinical samples, such as blood or saliva, leads to the formation of antigen-antibody complexes, causing visible color changes that can be detected with the naked eye or using simple portable readers. These gold nanoparticle-based LFAs offer rapid, sensitive, and cost-effective detection of viral infections, renders them apt for point-of-care testing in settings with constrained resources [119]. In these assays, gold nanoparticles are intricately functionalized with viral-specific antibodies or aptamers, enabling them to selectively bind to target viral antigens present in clinical samples such as blood or saliva. Upon interaction with viral antigens, the gold nanoparticles form antigen-antibody complexes, initiating a cascade of events that culminate in visible color changes. These changes are easily discernible to the naked eye or can be quantified using portable readers, facilitating rapid and on-site diagnosis of viral infections [120]. The beauty of gold nanoparticle-based LFAs lies in their simplicity, sensitivity, and cost-effectiveness. These assays offer a streamlined approach to viral detection, bypassing the need for sophisticated laboratory equipment and lengthy processing times [121]. Their rapid turnaround time and ease of use make them particularly suitable for point-of-care testing, especially in resource-limited settings where access to centralized laboratory facilities may be limited. Furthermore, the versatility of gold nanoparticles allows for the customization of LFAs to target specific viral strains or biomarkers, enabling tailored diagnostic solutions for different infectious diseases [122]. Additionally, ongoing research efforts continue to enhance the sensitivity and specificity of gold nanoparticle-based assays, paving the way for further advancements in viral detection and surveillance. In essence, gold nanoparticle-based assays represent a paradigm shift in viral diagnostics, offering a potent combination of speed, sensitivity, and simplicity [123]. As the field continues to evolve, these assays hold immense promise for revolutionizing infectious disease management and improving patient outcomes on a global scale.

Magnetic Nanoparticle-Based Nucleic Acid Detection

Magnetic nanoparticles have emerged as valuable tools for the detection of viral nucleic acids in clinical samples. One example is the use of magnetic nanoparticle-based nucleic acid extraction and amplification techniques for the diagnosis of respiratory viral infections, including influenza and respiratory syncytial virus (RSV) [124]. In these assays, magnetic nanoparticles functionalized with viral-specific probes are used to capture and concentrate viral RNA or DNA from patient samples [125]. Subsequent nucleic acid amplification steps, such as polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP), enable the sensitive and specific detection of viral genetic material [125]. These magnetic nanoparticle-based assays offer rapid turnaround times and high sensitivity, making them valuable tools for early diagnosis and surveillance of viral outbreaks. In the context of respiratory viral infections such as influenza and respiratory syncytial virus (RSV), magnetic nanoparticle-based assays play a crucial role in early diagnosis and surveillance of viral outbreaks [126]. The workflow typically begins with the functionalization of magnetic nanoparticles with viral-specific probes, such as oligonucleotide sequences complementary to target viral RNA or DNA sequences. These functionalized magnetic nanoparticles are then added to patient samples, where they selectively bind and capture viral nucleic acids of interest. Following nucleic acid capture, magnetic separation techniques are employed to isolate the magnetic nanoparticle-viral nucleic acid complexes from the sample matrix. This step allows for the efficient concentration and purification of viral genetic material, enhancing the sensitivity of downstream detection methods [127]. Subsequent nucleic acid amplification steps, such as polymerase chain reaction (PCR) or loop-mediated isothermal amplification (LAMP), are then performed to exponentially amplify the target viral nucleic acids, enabling their detection at clinically relevant levels. A notable benefit of nucleic acid detection using magnetic nanoparticles is its swift turnaround time, which is crucial for timely diagnosis and surveillance of viral outbreaks. Additionally, these assays offer exceptional sensitivity and specificity, allowing for the detection of low-abundance viral genetic material with high accuracy. Furthermore, magnetic nanoparticle-based assays can be easily adapted for high-throughput screening applications, making them suitable for large-scale epidemiological studies and surveillance efforts [128]. Overall, magnetic nanoparticle-based nucleic acid detection holds tremendous promise for advancing the field of viral diagnostics, particularly in the context of infectious diseases. By leveraging the unique properties of magnetic nanoparticles, these assays offer a robust and efficient platform for early detection, surveillance, and management of viral outbreaks, ultimately contributing to improved patient care and public health outcomes.

Quantum Dot-Based Immunoassays for Bacterial Biomarkers

Quantum dots (QDs) are semiconductor nanoparticles that exhibit unique optical properties, including size-tunable emission spectra and high photostability. QDs have been utilized in the development of highly sensitive immunoassays for the detection of bacterial biomarkers in infectious diseases [129]. In these assays, QDs conjugated with specific antibodies recognize and bind to the target toxins, leading to fluorescent signals that can be quantified using fluorescence spectroscopy or imaging techniques. Quantum dot-based immunoassays offer excellent sensitivity and multiplexing capabilities, enabling the simultaneous detection of multiple bacterial biomarkers in complex clinical samples [130]. In the context of infectious diseases, researchers have developed quantum dot-based immunoassays for the detection of bacterial toxins, such as staphylococcal enterotoxin B (SEB) and cholera toxin, which serve as crucial biomarkers for bacterial infections caused by pathogens like Staphylococcus aureus and Vibrio cholerae, respectively [131]. In these assays, quantum dots are conjugated with specific antibodies or aptamers that selectively recognize and bind to the target bacterial toxins with high affinity and specificity. Upon binding to the target toxins, the quantum dots emit fluorescent signals that can be quantified using fluorescence spectroscopy or imaging techniques [132]. The intensity of the fluorescent signal is directly proportional to the concentration of the target toxin in the sample, enabling precise and quantitative detection of bacterial biomarkers. Importantly, quantum dot-based immunoassays offer superior sensitivity compared to conventional detection methods, allowing for the detection of low concentrations of bacterial toxins in complex clinical samples. The multiplexing capability stands out as a major advantage of quantum dot-based immunoassays, which enables the simultaneous detection of multiple bacterial biomarkers within the same sample. By conjugating different types of quantum dots with antibodies or aptamers targeting distinct bacterial toxins, researchers can create multiplexed assays capable of detecting multiple pathogens or virulence factors in a single assay run. This multiplexing capability not only enhances the efficiency of diagnostic testing but also provides a more comprehensive assessment of bacterial infections, facilitating rapid and accurate diagnosis [133]. Furthermore, quantum dot-based immunoassays offer excellent photostability, allowing for prolonged and repeated imaging without signal degradation. This feature is particularly advantageous for longitudinal studies or high-throughput screening applications where sample stability and signal reliability are critical [134]. Overall, quantum dot-based immunoassays represent a powerful tool for the sensitive, specific, and multiplexed detection of bacterial biomarkers in infectious diseases. These assays hold great promise for improving diagnostic accuracy, enabling early detection of bacterial infections, and informing targeted therapeutic interventions, ultimately contributing to improved patient outcomes in the field of infectious disease management.

Nanoparticle-Based Multiplexed Immunoassays for Host Biomarkers

Nanoparticle-based multiplexed immunoassays have been developed for the simultaneous detection of host biomarkers indicative of immune responses or disease severity in infectious diseases [135]. For instance, researchers have utilized color-coded microspheres or magnetic nanoparticles functionalized with antibodies against different cytokines, chemokines, and acute-phase proteins to quantify multiple immune mediators in patient samples. These multiplexed assays enable the comprehensive profiling of host immune responses during infection, providing valuable insights into disease pathogenesis, treatment response, and patient prognosis [136]. Multiplexed nanoparticle-based immunoassays offer advantages such as high throughput, reduced sample volume requirements, and enhanced analytical sensitivity compared to conventional single-plex assays, making them valuable tools for infectious disease research and clinical management researchers employ a panel of nanoparticle-conjugated antibodies, each designed to recognize a distinct immune mediator relevant to the infectious disease under investigation [137]. These nanoparticles are then incubated with patient samples, such as blood or serum, allowing the antibodies to capture their respective target biomarkers present in the sample. Following incubation, the captured biomarkers are typically detected and quantified using various detection methods, such as fluorescence spectroscopy, flow cytometry, or magnetic resonance imaging (MRI) [138]. One key advantage of nanoparticle-based multiplexed immunoassays is their ability to simultaneously measure multiple immune mediators within a single sample. This enables researchers and clinicians to obtain a comprehensive profile of the host immune response, including the levels of pro-inflammatory cytokines, chemotactic factors, and markers of tissue damage or repair [139]. By analyzing this multi-dimensional immune profile, researchers can gain valuable insights into disease pathogenesis, identify potential biomarkers of disease severity or treatment response, and tailor therapeutic interventions accordingly. Moreover, nanoparticle-based multiplexed immunoassays offer several technical advantages over conventional single-plex assays. These include increased throughput, as multiple analytes can be measured in parallel using the same sample volume, thereby conserving precious clinical samples and reducing assay time and cost [140]. Additionally, the use of nanoparticles as detection probes enhances the analytical sensitivity of the assay, allowing for the detection of low-abundance biomarkers that may be missed by traditional assays.

3.4 Innovation in Molecular Diagnostics

Innovation in molecular diagnostics has revolutionized the landscape of infectious disease management, particularly in the nanomedicine era. Molecular diagnostics entail the detection and analysis of nucleic acids, proteins, and other biomolecules at the molecular level, allowing for precise identification of infectious agents and characterization of host responses [141]. With advancements in nanotechnology, these diagnostic approaches have undergone remarkable transformations, offering unprecedented sensitivity, specificity, and multiplexing capabilities. One of the key innovations in molecular diagnostics is the development of nucleic acid amplification techniques, such as polymerase chain reaction (PCR) [142] and loop-mediated isothermal amplification (LAMP). These methods enable the rapid and exponential amplification of target nucleic acids from infectious agents, providing highly sensitive and specific detection even at low concentrations. Moreover, the integration of nanomaterials, such as magnetic nanoparticles or gold nanoparticles, enhances the efficiency of nucleic acid extraction, amplification, and detection, further improving the performance of molecular diagnostic assays. Another significant innovation is the emergence of point-of-care molecular diagnostic devices that enable rapid and on-site testing for infectious diseases [143]. These portable devices leverage microfluidic technologies and miniaturized instrumentation to perform nucleic acid amplification and detection in a compact and user-friendly format. Nanoparticle-based detection strategies, such as lateral flow assays and nanoparticle-enhanced PCR, have been integrated into these devices to achieve rapid turnaround times and high analytical sensitivity, making them invaluable tools for early diagnosis and surveillance of infectious pathogens. Furthermore, multiplexed molecular diagnostic assays have revolutionized infectious disease diagnostics by enabling the simultaneous detection of multiple pathogens or biomarkers within a single sample. Nanoparticle-based multiplexing platforms, such as quantum dot-based immunoassays and magnetic nanoparticle-based nucleic acid detection assays, offer the ability to detect and quantify multiple targets with high specificity and sensitivity [144]. These multiplexed assays provide comprehensive insights into the complex interactions between pathogens and host immune responses, facilitating more accurate diagnosis, prognosis, and treatment monitoring. In addition to pathogen detection, molecular diagnostics have also advanced our understanding of antimicrobial resistance mechanisms and host-pathogen interactions. Innovative techniques, such as whole-genome sequencing and metagenomic analysis, enable comprehensive genomic profiling of infectious agents and their resistance determinants, guiding targeted antimicrobial therapy and infection control strategies [145]. Moreover, the integration of artificial intelligence and machine learning algorithms into molecular diagnostic platforms facilitates data analysis and interpretation [146], enabling real-time decision-making and personalized treatment recommendations.

Nanoparticle-Enhanced PCR for Point-of-Care Testing

One example of innovation in molecular diagnostics is the integration of nanoparticles into PCR-based assays to enable rapid and sensitive detection of infectious agents at the point of care. For instance, researchers at Harvard University developed a nanoparticle-based PCR assay for the detection of HIV RNA in resource-limited settings. In this assay, gold nanoparticles were conjugated with HIV-specific DNA probes, which hybridize to the target RNA sequences during PCR amplification [121]. The presence of amplified HIV RNA leads to the aggregation of gold nanoparticles, resulting in a color change that can be visually detected with the naked eye. This nanoparticle-enhanced PCR assay offers a simple and cost-effective approach to HIV detection [147], making it suitable for use in low-resource settings where access to laboratory infrastructure is limited.

Multiplexed Immunoassays Using Quantum Dots

Quantum dot-based immunoassays represent another innovative approach in molecular diagnostics, allowing for the simultaneous detection of multiple biomarkers with high sensitivity and specificity. For example, researchers at Johns Hopkins University developed a multiplexed immunoassay using quantum dots for the detection of respiratory viruses, including influenza A, influenza B, and respiratory syncytial virus (RSV) [148]. In this assay, different types of quantum dots were conjugated with virus-specific antibodies, enabling the simultaneous detection of viral antigens in patient samples. The use of quantum dots as detection probes provided enhanced sensitivity and multiplexing capabilities compared to traditional immunoassays, facilitating rapid and accurate diagnosis of respiratory viral infections.

Nanomaterial-Based Biosensors for Antimicrobial Resistance Detection

Nanomaterial-based biosensors have emerged as powerful tools for the detection of antimicrobial resistance genes and mutations, enabling rapid screening of pathogens for antibiotic susceptibility. For instance, researchers at MIT developed a graphene-based biosensor for the detection of antibiotic-resistant bacteria. In this biosensor, graphene nanosheets were functionalized with DNA probes specific to antibiotic resistance genes, allowing for the selective capture and detection of target sequences in bacterial samples. The integration of graphene nanomaterials into the biosensor platform provided high sensitivity and specificity, enabling rapid identification of antibiotic-resistant pathogens and guiding appropriate antibiotic therapy [149].

Microfluidic Molecular Diagnostic Devices for Infectious Disease Surveillance

Microfluidic molecular diagnostic devices have revolutionized infectious disease surveillance by enabling rapid and on-site testing for a wide range of pathogens. For example, the "Lab-on-a-Chip" developed by researchers at Stanford University integrates microfluidic channels, nucleic acid amplification techniques, and nanoparticle-based detection methods for the rapid diagnosis of infectious diseases such as tuberculosis and malaria [150]. This portable device allows for sample-to-answer analysis within a single platform, eliminating the need for complex laboratory infrastructure and enabling decentralized testing in remote or resource-limited settings [151].

These examples highlight the diverse applications of nanotechnology in molecular diagnostics, demonstrating how innovative approaches are transforming our ability to diagnose and manage infectious diseases with precision and efficiency.

4 Therapeutic Applications

This section of the chapter explores the transformative potential of nanotechnology in delivering targeted therapies, enhancing drug efficacy, and overcoming challenges such as drug resistance. From nanoantibiotics and antiviral nanoparticles to advanced drug delivery systems and nanoparticle-based immunotherapies, this segment delves into the diverse array of therapeutic strategies (Fig. 3) facilitated by nanomedicine. Additionally, the discussion extends to gene therapy, combination therapy approaches, and the development of nanoparticle-based antimicrobial agents, highlighting the exciting advancements and future prospects in the field of nanotherapeutics.

4.1 Nano Antibiotics

Nano antibiotics represent a promising application of nanotechnology, utilizing the physical and chemical coupling of minute particles with antibiotics or synthetically produced pure antibiotic molecules, typically within the size range of ≤100 nm in at least one dimension. These nanostructures have distinct physicochemical features, such as a higher surface area to volume ratio, greater reactivity, and increased permeability [152]. As a result, they display superior pharmacokinetic profiles, longer circulation durations, and greater targeting capabilities compared to traditional antibiotics [153]. The capacity of nano antibiotics to precisely target infected areas while minimizing off-target effects is one of its main advantages. Various nanostructures, such as liposomes, polymeric nanoparticles, inorganic nanoparticles, and dendrimers, can be functionalized with particular ligands to enable selective

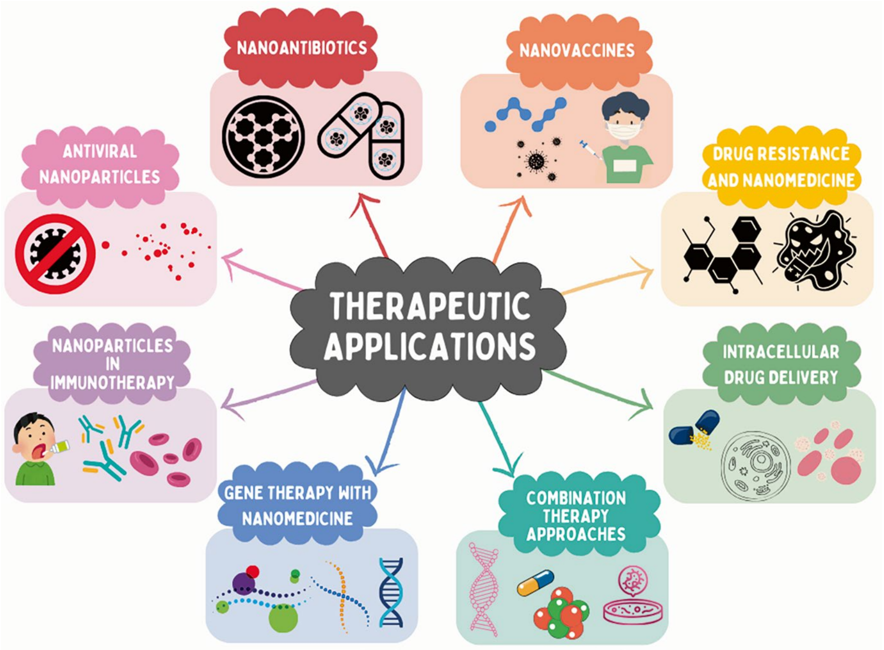
THERAPEUTIC APPLICATIONS
NANOANTIBIOTICS
NANOVACCINES
DRUG RESISTANCE AND NANOMEDICINE
INTRACELLULAR DRUG DELIVERY
COMBINATION THERAPY APPROACHES
GENE THERAPY WITH NANOMEDICINE
NANOPARTICLES IN IMMUNOTHERAPY
ANTIVIRAL NANOPARTICLES

Fig. 3 Therapeutic strategies in nanomedicine

binding to microbial surfaces and intracellular compartments. This targeted delivery strategy improves the therapeutic efficiency of antibiotics by assuring larger concentrations at the site of infection, therefore lowering the possibility of antimicrobial resistance and harmful effects on host tissues [154]. When it comes to combating a wide range of infections, including ones that are resistant to multiple drugs, nano antibiotics exhibit superior antibacterial efficacy. Their small size allows for efficient penetration of bacterial biofilms and cellular barriers, bypassing previous restrictions caused by poor diffusion or limited access to conventional antibiotics. Upon entering cells, nanoparticles either discharge antibiotics or interfere with bacterial metabolic pathways, exploiting a “Trojan horse” strategy to effectively combat infections [155]. Furthermore, nano formulations can be tailored to release antibiotics in a controlled manner, optimizing medication exposure while minimizing the formation of resistant bacterial subpopulations [156]. Moreover, in addition to standalone usage, the efficacy of nano antibiotics can be boosted by integrating them with other therapeutic agents or methods and overcome resistance [157]. Antibiotics doped on nanoparticles along with metal ions exhibit a strong nano-conjugation synergy, which facilitates various membrane permeabilization or efflux pump blocking, and inhibition of biofilm [158]. Additionally, nano formulations can facilitate the co-delivery of antibiotics with immunomodulatory drugs or diagnostic probes, allowing personalized and diverse therapy methods [159]. Fullerenes, carbon quantum dots and carbon materials, such as multi-wall carbon nano tubes that have also been investigated by several researchers for improved targeted specific delivery and resistance generation of microbes from antibiotics [160, 161]. Although nano antibiotics show great promise, a number of obstacles need to be overcome before they may be used in clinical settings. These include issues about biocompatibility, long-term safety profiles, production scalability, and regulatory approval procedures [153]. On top of that, the emergence of nanomaterial-specific toxicity and the potential for environmental impact requires comprehensive risk assessment and mitigation strategies [152]. Table 10 provides a comprehensive overview of various types of nano antibiotics and their respective mechanisms of action.

4.2 Antiviral Nanoparticles

As like nano antibiotics, antiviral nanoparticles are also an important part of nanomedicine, providing novel ways for treating viral infection. Most of the anti-retro viral and non-retro viral drugs have been exploited nanotechnology field for efficient therapeutic outcomes [172]. These nanoparticles, made from a range of nanomaterials, have unique features that allow for targeted interactions with viral infections [173]. They interfere with viral entrance, impede replication, induce host immunological responses, and impair viral assembly and release [174]. Metal nanoparticles, such as silver, iron oxide, gold, and copper, have natural antiviral characteristics and can be functionalized with antiviral drugs to improve potency [175–178]. Liposomes and lipid nanoparticles are efficient carriers of antiviral medicines or nucleic acids, allowing for targeted delivery and prolonged release [179]. Polymeric nanoparticles such as chitosan, PLGA, and dendrimers are versatile in terms of design and surface modification, allowing for specific antiviral applications [180–182]. High concentrations of high molecular weight chitosan can able to prevent infection from murine norovirus (MNV-1) [183, 184]. Furthermore, carbon-based nanomaterials, such as carbon nanotubes and graphene oxide, show antiviral activity through interactions with viral components [185, 186]. Antiviral nanoparticles offer a wide range of uses in viral infection management, including surface coatings, air filtration systems, and disinfection agents that prevent viral transmission. They also show potential as therapeutic interventions, offering targeted and sustained administration of antiviral medicines or vaccinations while minimizing side effects [187]. Furthermore, these nanoparticles act as diagnostic instruments, detecting viral infections in a sensitive and precise manner, allowing for rapid diagnosis and surveillance. Antiviral nanoparticles provide a flexible framework for quick response and pandemic readiness in the face of new viral threats [188]. Looking ahead at the future of antiviral medications, it’s crucial for us to focus on some key aspects. Firstly, we need to develop nanoparticles that can act like little receptors, safely targeting specific viruses in our bodies. Think of them as tiny, smart scouts seeking out and neutralizing harmful viruses swiftly and effectively. Secondly, these nanoparticles should be tailored with specific molecules to make sure they hit the right spots where viral infections occur. It’s like giving them a GPS

Table 10 Different types of nano antibiotics and their functioning

Nanoparticle	Size/shape	Conjugated antibiotic	Targeted bacteria	Target site	Mechanism of action	Reference
Metal oxide nanoparticles- CeO_2	5–20 nm; spherical	B-lactam antibiotics	*K. pneumoniae* *E. coli*	Outer cell membrane	Increases the outer cell membrane's permeability by passively diffusing antibiotics; Ce^{3+} or Ce^{4+} ions cause oxidative stress on the cell membrane.	[162]
SPIONs (Superparamagnetic iron oxide nanoparticles)	25 nm; spherical	Vancomycin	*S. aureus* *B. subtilis* MRSA *S. aeruginosa*	Outer cell membrane	Cell membrane injury causes intracellular protein and lactate dehydrogenase leaks, as well as the production of oxygen-free radicals and impairment of mitochondrial activities. Vancomycin is delivered slowly and continuously, requiring lower amounts to establish antibacterial activity and reducing the likelihood of antibiotic resistance development.	[163]
Graphene oxide nanoparticles	200 nm; sheet	Vancomycin	VRSA	Cell wall and cell membrane	After being released and interacting with negatively charged bacteria, the vancomycin-graphene oxide conjugate can release a sizable amount of cationic VAN. Reactive oxygen species are produced as a result, increasing turgor pressure and damaging membranes. Thus, there is an inhibition of cell wall shrinkage, which ultimately results in cell lysis.	[164]
Single walled carbon nanotubes (SWCNTs)	500 nm; cylindrical	Tetracyclin (TET)	*E. coli*	Membrane proteins	Needle-shaped SWCNTs penetrate bacterial barriers more effectively, delivering TET into the cytoplasm to bind with ribosomes more efficiently. The size of SWCNTs exceeds that of efflux pump membrane proteins, allowing TET to be retained inside the cell, thereby inhibiting bacterial growth.	[165]

(continued)

Table 10 (continued)

Nanoparticle	Size/shape	Conjugated antibiotic	Targeted bacteria	Target site	Mechanism of action	Reference
Graphene oxide nanoparticles	600 nm; flakes	Linezolid (LZD)	*M. tuberculosis*	Macrophages in alveoli	LZD keeps working effectively because it doesn't stick well to graphene oxide (GO) surfaces, making it easy to release inside the infection site. GO helps LZD to gather more in the lungs and stops *M. tuberculosis* from getting into the macrophages, which lowers the infection. When GO and LZD are given together, they work even better against TB and might stop antibiotic resistance from happening. Combination of GO with LZD produces two to three times more reactive oxygen species (ROS) compared to just using LZD.	[166]
Polymeric nanoparticle (PLGA)	50–500 nm; spherical	Azithromycin, Rifampin	*C. trachomatis*	Live infected cells	Dual mechanisms of action involved: firstly, the combined azithromycin and rifampin therapy demonstrates improved effectiveness in reducing the burden of chlamydia. Secondly, there is sustained release of drugs at both acute and persistent infection sites.	[167]
Polymeric nanoparticle (PLGA)	150–250 nm; spherical	Vancomycin	*E. coli*	Cell wall	By the modification of surface charge under influence of acidic pH (pH 5.5–6.0), NPs can modify to positive charge and it causes the strongly attachment to bacterial cell wall by making strong multivalent electrostatic binding force. This enhances antibacterial activity and sustained release of vancomycin	[168]

Mesoporous silica	50–90 nm; spheroid	Vancomycin, Polymyxin B	*S. aureus* *P. aeruginosa* *E. coli*	Outer cell membrane	The combined action of polymyxin B and vancomycin enhances the potency of antibiotics against both gram-positive and gram-negative bacteria. Polymyxin B targets the outer membrane of gram-negative bacteria, while vancomycin disrupts peptidoglycan synthesis. Carboxyl-loaded mesoporous silica nanoparticles (MSNs) with a lower release rate, attributed to their higher net negative charge, amplify antibacterial efficacy by increasing the local concentration of immobilized antibiotics.	[169]
Mesoporous silica with Cu(II) and Ni(II) complexes	Less than 100 nm; spherical	Gentamicin	*S. aureus* *B. subtilis* *P. aeruginosa* *E. coli*	Cell membrane	Small porous structure of metal-mesoporous silica nanostructures enables greater loading of and increased release of gentamicin in the cell membrane. It also enables higher enzyme immobilization.	[170]
Solid lipid nanoparticles	150–300 nm; spherical	Levofloxacin, Penicillin G	*MRSA*	Cell membrane	Nanoparticles can improve the internalization of both drugs into the cell membrane and increases bactericidal activity.	[171]

to navigate directly to the trouble zones in our bodies. Thirdly, we have to ensure that these treatments are gentle on our bodies, causing little to no harm. It's important that they're biocompatible and don't have any unpleasant side effects [189]. While viruses hold considerable potential for applications in nanotechnology, plant viruses offer distinct advantages. Unlike some viruses that affect animals, plant viruses are non-pathogenic. And also they feature non-infectious capsids known as empty virus-like particles (eVLPs), which can be efficiently replicated in large quantities using plants as hosts. This method of production is generally regarded as secure and cost-effective compared to alternative host-expression systems such as yeast, insect cells, and bacteria. Various nanomaterials and nanostructures are commonly developed using virus-like particles (VLPs) derived from icosahedral plant viruses like Cowpea mosaic virus (CPMV), Tobacco mosaic virus (TMV), Brome mosaic virus (BMV), and Hibiscus chlorotic ringspot virus (HCRSV) [190–193]. Moving forward, we should investigate therapeutic strategies that combine these nanoparticles with modest dosages of antiviral medications. It's like combining a surgeon's accuracy with a warrior's firepower: powerful but exact. Finally, to address the intricacies of viruses, their mutations, and the problems of administering antiviral medications during pandemics, we must bring together experts from many domains. By developing groups with various talents and knowledge, we can better understand and solve these concerns, ensuring that our efforts to combat infections are successful and ethical.

4.3 Nano Vaccines

Throughout history, the field of vaccination has been shown significant progressions. Following Jenner's pioneering smallpox vaccine, Louis Pasteur focused on isolating and weakening disease-causing microbes to provide immunity. This method successfully immunized against cholera, rabies, and anthrax. Conventional vaccines are having some disadvantages including less immunogenicity, in vivo instability, toxicity and need of multiple dosages. Integrating nanotechnology field into vaccine development can be able to make enhanced delivery, improved immunogenicity, and prolonged efficacy [194]. Specifically, delivery systems can optimize immunological outcomes in several ways. Firstly, they can extend the presence of antigens at the administration site, ensuring prolonged exposure to the immune system. Secondly, they facilitate the recruitment of sentinel immune cells, known as antigen-presenting cells (APCs), which are crucial for initiating immune responses. Thirdly, they influence the localization and delivery of antigens to specific sites within the body. Lastly, they safeguard fragile payloads, such as nucleic acids, from degradation or premature release. When exploring various carriers for vaccine delivery, liposomes emerge as the most extensively researched and impactful nanoparticles for effectively delivering vaccines. Liposomes offer several advantages such as biocompatibility, versatility, and the ability to encapsulate various antigens and adjuvants. Abhyankar and colleagues (2018) synthesized liposomes as adjuvants that included synthetic Toll-like receptor (TLR) ligands, GLA and 3M-052, in addition to co-administering LecA, a putative antigen against *Entamoebahistolytica*-caused amebiasis [195]. Current examples of liposome-based nano vaccines include the influenza vaccine FluMist®, which utilizes liposomal delivery to enhance mucosal immune responses, and the hepatitis B vaccine Engerix-B®, which incorporates liposomes to improve antigen stability and immunogenicity [196, 197]. With ongoing exploration and refinement in liposome-based nano vaccines, there is potential to revolutionize preventive healthcare by providing safer, more effective and personalized immunization strategies against a wide range of viruses and diseases. Gold nanoparticles (AuNPs) have garnered significant attention in the field of vaccinology because of their dependable surface modification, compatibility with biological systems, and the ability to customize their size, shape, and optical characteristics. Their inert nature allows for utilization as both delivery vehicles and adjuvants, facilitating substantial enhancement of immune responses while ensuring minimal toxicity [198]. From ex vivo studies, researchers have been shown that the AuNPs are capable to increase the proliferation of HIV specific $CD4^+$ and $CD8^+$ and induction of secretion of highly active cytokines [199]. Administering low molecular weight polyinosinic-polycytidylic acid (uPIC) coated gold nanorods intranasally without needles, in combination with influenza virus hemagglutinin, demonstrated increased adjuvant effectiveness of uPIC (40:400). This resulted in the inhibition of influenza viral infection in mice while minimizing the production of inflammatory cytokines [200]. Another notable application of gold-based nanovaccines is in cancer immunotherapy. Gold nanoparticles can be conjugated with tumor antigens and immune-stimulating molecules to create cancer vaccines. These nanovaccines are designed to target tumor cells specifically, stimulating the immune system to identify and eliminate cancerous cells. Clinical trials are underway to evaluate the efficacy of gold-based nanovaccines in various types of cancer, including melanoma and breast cancer [201, 202]. FDA approved linear co-polymer of lactic and glycolic acid- PLGA- is also an important vector to bring out vaccination process. Nanovaccines using PLGA as a base provide the opportunity to simultaneously deliver various antigens or immunomodulators, enabling the creation of multivalent vaccines that target multiple pathogens or strains of a disease. This method simplifies vaccine administration and enhances protection against a wide range of microbial dangers [203]. A multitude of PLGA-based nanovaccines have demonstrated promise in preclinical and clinical studies for a variety of infectious diseases, including human papillomavirus (HPV), influenza, and malaria. Comparing these novel vaccines to conventional vaccination formulations, researchers have found that the former have increased immune response activation, increased protective capacity, and decreased side responses [204–207]. Silica-based, carbon nanotube-based (CNT), nanogel-based, chitosan-based, and virus-like particle-based (VLP) nanovaccines are novel approaches to vaccine development. Each type of nanovaccine provides distinct advantages in terms of antigen delivery, immunological activation, and protective efficacy, helping to develop preventive medicine against infectious illnesses and malignancies. Studies have been conducted by mesoporous silica nanoparticles (MSN) as adjuvants in a vaccine incorporating recombinant H1N1 influenza hemagglutinin antigen (HAC1), generated through genetically modified plants [208]. Functionalized CNTs can effectively transfer antigens and adjuvants to immune cells, resulting in strong immunological responses. For example, CNT-based nanovaccines have been created for cancer immunotherapy, where they improve antigen presentation and activate cytotoxic T lymphocyte responses against tumour cells [209]. Three dimensional network structures of nano gels are offering high loading capacity and stability to vaccine components. These nanovaccines can be designed to deliver antigens and adjuvants in a regulated manner, thereby boosting immune responses. Nanogel-based vaccines have been created for infectious diseases such as HIV and malaria, where they increase antigen presentation and promote long-term immunological memory [210, 211]. Scientists have created chitosan-based nanovaccines specifically for combating respiratory infections such as influenza and bacterial threats like *Streptococcus pneumoniae*. These advancements have shown to booster mucosal immunity and provide increased protection against infections [212]. VLP-based nanovaccines have been successfully developed for various infectious diseases, including human papillomavirus (HPV), hepatitis B virus (HBV), and Ebola virus. These nanovaccines can induce potent immune responses, including both humoral and cellular immunity, leading to durable protection against viral infections [213–215]. A recent study demonstrated the use of a metal-organic framework (MOF) nanoparticle, which can deliver vaccines and simultaneously act as an adjuvant to strengthen the immune response [216]. Another example is the NVX-CoV2373 vaccine, which utilizes spike protein nanoparticles to stimulate an immune response against COVID-19 [217]. These nanoparticles are preferentially taken up by antigen-presenting cells, which are crucial for initiating an immune response. Moreover, the versatility of nanoparticles allows for the development of vaccines that can potentially provide immunity against multiple viruses, showcasing the potential of nanotechnology in advancing vaccine efficacy and safety.

4.4 Drug Resistance and Nano Medicine

Drug resistance presents a serious problem for the medical field since it allows bacteria to evolve defence mechanisms against the effects of traditional treatments, which can result in treatment failures and higher death rates. Antibiotic resistance manifests through various mechanisms, including alterations at the molecular and epitopic levels on the microorganism’s surface, chromosomal mutations, enzymatic deactivation, antibiotic transport outside the cell, and activities within the periplasmic space [218]. Nanomedicine presents a viable answer to drug resistance by introducing novel ways to drug delivery, targeting, and therapy. One example of nanomedicine addressing drug resistance is the use of nanoparticle-based delivery systems to overcome bacterial resistance. Antibiotic-resistant bacteria, such as Methicillin-resistant Staphylococcus aureus (MRSA), present a serious threat to public health [219]. Nanoparticles loaded with antibiotics can bypass bacterial resistance mechanisms, allowing for effective targeting and delivery of antimicrobial agents to infected sites. This approach has shown promising results in enhancing the efficacy of antibiotics against drug-resistant bacterial infections. As we previously noted, inorganic nanoparticles including AgNPs, titanium based nanoparticles are having the ability to quickly adhere and accumulate on microbial cell walls, followed by penetration through pores due to their small size [220]. They can overcome bacterial resistance mechanisms by targeting multiple cellular pathways. And also nanovaccines are designed to elicit broad-spectrum immune responses can help prevent the emergence of drug-resistant strains by reducing the reliance on antibiotics for disease treatment. MSN based nanovaccine have been successfully protected experimental mice from *P. aeruginosa* resistant strains [221]. Nanoparticles containing host defense peptides (antimicrobial peptides—AMPs) are also utilized to combat infections caused by multidrug-resistant organisms. AMPs are more effective bactericidal than normal antibiotics, because they will make strong electrostatic interaction with microbial membrane [222]. When bacteriocin, a ribosome produced peptide was administered via a nano-drug delivery system, its antibacterial properties improved. For increased efficacy, the NPs carrying peptides could be tailored to target the affected area precisely [223]. Similarly, nanomedicine has been applied to combat cancer drug resistance. Tumor cells commonly develop resistance to chemotherapy drugs, which often leads to treatment failures and the progression of the disease [222]. Nanoparticle-based drug delivery systems can improve the bioavailability and tumor accumulation of chemotherapeutic agents, overcoming resistance mechanisms such as efflux pumps and drug metabolism. For example, nanoparticle formulations of anticancer drugs like doxorubicin and paclitaxel have demonstrated enhanced therapeutic efficacy and reduced drug resistance in preclinical studies and clinical trials [224, 225]. Nanomaterial-based photothermal and photodynamic therapies present intriguing approaches to counter drug resistance in various medicinal applications. These methods use the special qualities of nanoparticles to administer focused therapies that get beyond resistance mechanisms [226]. Photothermal therapy involves the use of light-absorbing nanomaterials, such as gold nanoparticles or carbon nanotubes, which convert absorbed light into heat. When these nanomaterials are delivered to the site of infection or cancer and irradiated with light of a specific wavelength, they generate localized heat, leading to the destruction of drug-resistant pathogens or cancer cells. Importantly, this heat-based approach can circumvent resistance mechanisms that render conventional antibiotics or chemotherapeutic agents ineffective [227]. On the other hand, photodynamic therapy relies on photosensitizing nanomaterials, such as quantum dots or organic dyes, which generate reactive oxygen species (ROS) upon light irradiation. These ROS are highly cytotoxic and can induce cell death in drug-resistant pathogens or cancer cells. Moreover, photodynamic therapy can overcome resistance mechanisms by targeting multiple cellular pathways simultaneously, making it difficult for pathogens or cancer cells to develop resistance [228, 229]. Photothermal and photodynamic therapies present numerous benefits in addressing drug resistance. They offer precise spatial and temporal control over treatment, thereby reducing harm to surrounding healthy tissues. They can be used in combination with traditional therapies to boost their effectiveness and overcome resistance [230]. These therapies can also be customized to target specific pathogens or cancer cells, leading to enhanced treatment outcomes. Continued research and advancement in this area are needed to make the potential transformation in treatment methods and enhance patient outcomes in combating drug-resistant infections and cancers.

4.5 *Intracellular Drug Delivery*

Delivering drug components into the cellular level is the toughest part due to the presence of the plasma membrane. This biological barrier separates the interior of the cell from the outer environment. Nanoparticles are capable of bypassing this biological barrier based on their size, shape, elasticity and surface charge. Nanomedicine utilizes nanoparticles as carriers to transport drugs across cellular barriers and deliver them to specific intracellular targets. There are different kinds of cellular mechanisms available to bypass this unique lipid bilayer, thereby achieving intracellular drug delivery [231]. Generally, there are three types of mechanisms, namely, endocytosis, receptor-based uptake pathways and direct cellular uptake pathways [232]. Endocytosis-mediated intracellular drug delivery is a sophisticated process by which cells internalize substances from their external environment through the formation of vesicles. This mechanism is widely exploited in nanomedicine to deliver therapeutic agents into cells for the treatment of various diseases. Phagocytosis, pinocytosis, and receptor-mediated endocytosis are among the several processes that make up endocytosis. In phagocytosis, cells engulf large particles, while pinocytosis involves the uptake of fluids and solutes. Molecules attach to cell surface receptors through a highly precise mechanism called receptor-mediated endocytosis, which allows the molecules to be internalized by clathrin-coated vesicles [233]. Nanoparticles designed for drug delivery can exploit endocytosis mechanisms to enter cells. For example, nanoparticles coated with ligands that bind to cell surface receptors can undergo receptor-mediated endocytosis, resulting in their internalization into cells. Additionally, nanoparticles of appropriate size and surface charge can be engulfed by cells through phagocytosis or pinocytosis [232]. Liposomes, lipid-based nanoparticles, are employed in cancer therapy as they utilize receptor-mediated endocytosis for targeted drug delivery. Encapsulating chemotherapy medications within liposomes enables specific targeting of cancer cells. Once taken up by the cancer cells through endocytosis, the liposomes release the drug intracellularly, leading to cell death. Liposomal doxorubicin, a chemotherapy variant, functions based on this principle by specifically targeting cancerous cells while minimizing harm to healthy tissues [232]. Similarly, Nucleic acid therapies, like siRNA or mRNA, can be delivered for gene therapy using polymer-based nanoparticles that go through endocytosis. These nanoparticles carry siRNA molecules into cells to silence genes. They shield siRNA from breaking down and help it enter cells. Once inside, siRNA is released from the nanoparticles and enters the RNA interference pathway, targeting specific mRNA molecules to stop their action [234]. Researchers employ various strategies to optimize the efficiency of endocytosis-mediated drug delivery. This includes modifying nanoparticle properties, such as size, surface charge, and surface functionalization, to enhance their uptake by cells. Additionally, the use of targeting ligands, such as antibodies or peptides, can improve the specificity of nanoparticle uptake via receptor-mediated endocytosis [235]. Despite its potential, endocytosis-mediated drug delivery faces challenges, such as endosomal escape and intracellular trafficking. Strategies to enhance endosomal escape, such as pH-sensitive nanoparticles or membrane-disrupting agents, are actively being pursued to improve drug delivery efficiency [236]. Targeted administration of therapeutic substances into cells is made easier by a complex method called receptor-mediated intracellular drug delivery, which takes advantage of the body's innate receptor-ligand connections. Improved therapy results, decreased off-target effects, and increased specificity are just a few benefits of this approach. Ligands, such as antibodies or peptides, bind to specific cell surface receptors to enable receptor-mediated intracellular drug delivery. Following ligand-receptor engagement, the receptor-ligand complex internalizes the drug-loaded carrier into the cell through vesicular transport by going through endocytosis [237]. Antibody drug conjugate (ADC) is an example of receptor mediated intracellular drug delivery. ADCs are a class of targeted therapies that consist of monoclonal antibodies conjugated to cytotoxic drugs. Upon binding to cell surface receptors overexpressed on cancer cells, such as HER2 or CD20, ADCs are internalized via receptor-mediated endocytosis, delivering the cytotoxic payload directly into cancer cells while sparing healthy tissues [238]. Transferrin receptors are another example of receptors capable of delivering drugs into cells. They are frequently upregulated on the surface of cancer cells to fulfill their heightened need for iron. Nanoparticles functionalized with transferrin ligands can precisely target cancer cells through transferrin receptor-mediated endocytosis, allowing for the intracellular delivery of chemotherapeutic agents or imaging probes [239]. Folate receptors are also upregulated highly in cancer cells than healthy normal cells. Folate-conjugated nanoparticles or liposomes can selectively target cancer cells by binding to folate receptors, facilitating the intracellular delivery of anticancer drugs or nucleic acid therapeutics [240, 241]. Hormone receptors, such as estrogen receptors (ER) and androgen receptors (AR), play crucial roles in hormone-dependent cancers like breast and prostate cancer. Hormone receptor-targeted therapies, including hormone receptor antagonists or agonists, can be delivered intracellularly using receptor-mediated mechanisms to modulate cancer cell growth and survival. When it comes to optimizing drug release within target cells and minimizing off-target effects and systemic toxicity, receptor-mediated drug delivery methods provide enhanced safety and therapeutic efficacy [242]. Variability in receptor expression levels among patient populations and within tumors can affect the efficacy of receptor-mediated drug delivery. Efforts are underway to enhance endosomal escape strategies, improving drug release efficiency. Future research may focus on combining receptor-mediated drug delivery systems with complementary therapies like immunotherapy or gene editing for synergistic effects in disease treatment [243]. Direct cellular uptake of nanomedicine entails the targeted transport of therapeutic substances into the cytoplasm of cells, bypassing biological barriers such as the plasma membrane and endosomes. This technique has substantial advantages for delivering medicines, nucleic acids, and other bioactive substances to intracellular targets [244]. The conjugation of cell-penetrating peptides (CPPs) to nanoparticles is a recognized example of direct cytoplasmic delivery. CPPs have the ability to traverse cellular membranes and facilitate the uptake of nanoparticles into the cytoplasm. For instance, in cancer therapy, CPP-conjugated nanoparticles loaded with chemotherapeutic medicines can rapidly enter tumor cells and release their cargo directly into the cytoplasm, leading to improved anticancer efficacy while limiting systemic toxicity. CPPs linked to nanoparticles are a well-known example of direct cytoplasmic administration. CPPs can pass through cellular membranes, allowing nanoparticles to enter the cytoplasm more easily. For example, in cancer treatment, CPP-conjugated nanoparticles loaded with chemotherapeutic medicines can rapidly enter tumor cells and release their cargo directly into the cytoplasm, leading to improved anticancer efficiency while minimizing systemic damage [245, 246]. Viral vectors developed for gene delivery can also be used to transfer nucleic acids directly into the cytoplasm. Viral vectors, such as adenoviruses and lentiviruses, have developed complex mechanisms for penetrating cellular membranes and delivering genetic payloads into the cell. Researchers have developed efficient gene delivery methods for gene therapy and vaccine development by leveraging the intrinsic features of viral vectors and altering their surface proteins [247, 248]. Gold nanoparticles are promising tools for photothermal therapy in cancer treatment. By functionalizing them with targeting ligands and loading them with photothermal agents, these nanoparticles can be directed to cancer cells. When exposed to near-infrared light, gold nanoparticles generate heat, causing localized hyperthermia and selective destruction of cancer cells, while sparing healthy tissues. This method enables precise delivery of therapeutic heat to tumor cells, offering a targeted approach to cancer therapy [249, 250]. Additionally, quantum dots, semiconductor nanoparticles, hold potential for both imaging and drug delivery applications in biomedicine. These nanoparticles can be conjugated with targeting ligands and loaded with imaging agents or drugs, allowing them to penetrate cellular membranes and deliver their cargo directly into cells. This capability facilitates intracellular imaging, drug delivery, and targeted therapy, offering versatile solutions for various biomedical challenges [251, 252]. Nanoparticles are delivered directly to cells using a combination of passive and active methods that allow them to pass through cellular barriers and enter the cell. Understanding these systems enables researchers to create nanoparticles with improved cellular uptake and intracellular transport capabilities for a variety of biological applications, such as the administration of drugs, gene therapy, and imaging.

4.6 Nanoparticles Based Antimicrobial Agents

In recent years, the emergence of antimicrobial resistance (AMR) has posed a significant threat to global public health. Traditional antibiotics are becoming increasingly ineffective against resistant pathogens, highlighting the urgent need for innovative therapeutic approaches [253]. Nanotechnology has emerged as a promising field in the fight against infectious diseases, offering novel solutions through the development of nanoparticle-based antimicrobial agents. Nanoparticles are particles with dimensions on the nanometer scale, typically ranging from 1 to 100 nm [254]. Due to their small size and large surface area-to-volume ratio, nanoparticles exhibit unique physicochemical properties that make them highly effective as antimicrobial agents. These properties include enhanced stability, increased bioavailability, and the ability to penetrate microbial biofilms [255]. Nanoparticle-based antimicrobial agents offer a significant advantage in their ability to effectively target a broad spectrum of pathogens, including bacteria, viruses, fungi, and parasites. Various types of nanoparticles, such as metal nanoparticles (e.g., silver, gold, copper), metal oxide nanoparticles (e.g., zinc oxide, titanium dioxide), and polymeric nanoparticles (Table 11), have demonstrated potent antimicrobial activity against multidrug-resistant pathogens [256]. Silver nanoparticles (AgNPs) have garnered significant attention as antimicrobial agents due to their unique physicochemical properties and potent antimicrobial activity against a broad spectrum of pathogens, including bacteria, viruses, and fungi [257]. Their antimicrobial effects are mediated through multiple mechanisms, which contribute to their efficacy in fighting microbial infections. Silver nanoparticles have been observed to engage with the phospholipid bilayer of microbial cell membranes. Due to their small size and high surface area-to-volume ratio, AgNPs can penetrate the cell membrane, leading to structural damage and destabilization [258]. This disruption compromises the integrity of the membrane, resulting in leakage of cellular contents and eventual cell death. Moreover, the presence of silver ions released from AgNPs further exacerbates membrane damage by interfering with membrane-bound proteins and ion channels [259]. These have been reported to inhibit key enzymes involved in microbial respiration, such as Cytochrome C oxidase and NADH dehydrogenase. By targeting these respiratory enzymes, AgNPs disrupt the electron transport chain and impair ATP synthesis, thereby depriving microbial cells of essential energy sources [260]. This metabolic disruption impairs microbial growth and viability, contributing to the antimicrobial effects of silver nanoparticles. They possess the ability to generate reactive oxygen species (ROS), such as superoxide radicals (O_2^-), hydroxyl radicals (OH^-), and singlet oxygen ($^1O_2$), through redox reactions on their surface. These ROS exert oxidative stress on microbial cells, leading to damage to DNA, proteins, and lipids [261]. The accumulation of ROS overwhelms the antioxidant defense mechanisms of microbes, resulting in oxidative damage and ultimately cell death. Additionally, the generation of ROS by AgNPs contributes to their broad-spectrum antimicrobial activity, as ROS can target multiple cellular components essential for microbial survival. Biofilms are structured communities of microbes encased in a self-produced extracellular matrix, which confers increased resistance to antimicrobial agents and host immune defenses [262]. Silver nanoparticles have been demonstrated to inhibit the formation and maturation of biofilms by interfering with quorum sensing, adhesion, and extracellular matrix production by microbial cells. Moreover, AgNPs can penetrate pre-formed biofilms and disrupt their structural integrity, making them more susceptible to antimicrobial treatments [263]. AgNPs can potentiate the antimicrobial effects of antibiotics by overcoming microbial resistance mechanisms, such as efflux pumps and biofilm formation [264]. Additionally, the synergistic interaction between AgNPs and antibiotics may reduce the required dosage of antibiotics, minimizing the risk of adverse effects and slowing the emergence of antibiotic resistance. Gold nanoparticles (AuNPs) have emerged as promising antimicrobial agents due to their unique properties and versatile applications [265]. Their antimicrobial mechanisms encompass multiple pathways, including the disruption of microbial membranes, inhibition of vital enzymes, and interference with microbial DNA replication and protein synthesis. AuNPs interact with microbial cell membranes, leading to structural damage and permeabilization, which disrupts membrane integrity and compromises cellular functions [266]. Additionally, AuNPs have been shown to inhibit key enzymes involved in microbial metabolism, such as ATP synthase and DNA polymerase, thereby impairing essential cellular processes. Moreover, the interaction of AuNPs with microbial DNA and proteins interferes with their replication and synthesis, further impeding microbial growth and viability [267]. Furthermore, the surface of gold nanoparticles can be functionalized with antimicrobial peptides or antibodies, enabling specific targeting of pathogens and enhancing their antimicrobial efficacy. This ability to tailor AuNPs for targeted delivery makes them attractive candidates for combating microbial infections, including those caused by multidrug-resistant pathogens [268]. Overall, the multifaceted antimicrobial mechanisms and functionalization capabilities of gold nanoparticles hold great promise for the development of effective antimicrobial therapies in the nanomedicine era. Metal oxide nanoparticles have emerged as promising antimicrobial agents alongside metal nanoparticles due to their unique properties and mechanisms of action. Zinc oxide nanoparticles (ZnO NPs) have shown strong antimicrobial effects against a broad spectrum of pathogens, encompassing bacteria, viruses, and fungi [269]. Their antimicrobial mechanisms involve multiple pathways, including the disruption of microbial membranes, inhibition of microbial enzyme activity, and induction of oxidative stress. ZnO NPs interact with microbial cell membranes, leading to structural damage and permeabilization, which disrupts membrane integrity and compromises cellular functions. Furthermore, ZnO NPs inhibit microbial enzyme activity, such as DNA replication and protein synthesis, thereby impairing essential cellular processes and inhibiting microbial growth [270]. Additionally, ZnO NPs induce oxidative stress by generating reactive oxygen species (ROS), such as superoxide radicals and hydroxyl radicals, which cause damage to microbial DNA, proteins, and lipids, ultimately leading to microbial cell death. Titanium dioxide nanoparticles (TiO_2 NPs) possess photocatalytic properties that enable them to generate ROS upon exposure to light. This photocatalytic activity enhances their antimicrobial efficacy by inducing oxidative stress and damage to microbial cells [271]. TiO_2 NPs have been widely used in disinfection applications, such as water treatment and surface sterilization, due to their ability to effectively eliminate microbial contaminants [272]. Overall, metal oxide nanoparticles, including ZnO NPs and TiO_2 NPs, hold great potential as antimicrobial agents for combating microbial infections and addressing challenges associated with antimicrobial resistance. Polymeric nanoparticles present a versatile and promising approach for addressing infectious diseases. These nanoparticles offer a multifaceted strategy by encapsulating various antimicrobial agents, including antibiotics, antimicrobial peptides, or essential oils, within their polymeric matrix [273]. This encapsulation enables controlled release kinetics, allowing for sustained and targeted delivery of antimicrobial agents to specific sites of infection. By controlling the release rate, polymeric nanoparticles can optimize therapeutic efficacy while minimizing adverse effects associated with systemic drug administration. Moreover, polymeric nanoparticles can be engineered with surface modifications to enhance their stability, biocompatibility, and targeting specificity. Surface modifications, such as the addition of targeting ligands or protective coatings, enable selective recognition and binding to microbial cells or infected tissues, thereby improving drug delivery efficiency and reducing off-target effects [274]. Additionally, surface modifications can enhance nanoparticle stability in biological environments and reduce clearance by the immune system, prolonging circulation time and enhancing therapeutic outcomes. Overall, polymeric nanoparticles represent a promising platform for combating infectious diseases by providing tailored drug delivery systems with enhanced efficacy, safety, and targeting capabilities.

Despite their immense potential, the clinical translation of nanoparticle-based antimicrobial agent faces several challenges, including concerns regarding their safety, biocompatibility, and long-term effects. Additionally, the development of scalable manufacturing processes and regulatory approval pathways remains crucial for their widespread adoption in clinical practice [275].

4.7 Nanoparticles in Immunotherapy

In recent years, immunotherapy has emerged as a groundbreaking approach for combating infectious diseases by harnessing the power of the immune system to recognize and eliminate pathogens. Nanoparticles have played a pivotal role in revolutionizing immunotherapy strategies, offering innovative platforms for targeted delivery of immunomodulatory agents, vaccines, and immune-stimulating adjuvants [276]. This chapter explores the multifaceted applications of nanoparticles in immunotherapy, highlighting their potential to transform the landscape of infectious disease management in the nanomedicine era.

Table 11 summarizes the roles of different nanoparticles as antimicrobial agents, showcasing their effectiveness in combating microbial infections

Nanoparticle type	Mechanisms of antimicrobial action	Additional features
Silver nanoparticles (AgNPs)	Disruption of microbial cell membranes, inhibition of respiratory enzymes, generation of reactive oxygen species (ROS)	Inhibition of biofilm formation, enhancement of existing antibiotic activity
Gold nanoparticles (AuNPs)	Disruption of microbial cell membranes, inhibition of vital enzymes, interference with microbial DNA replication and protein synthesis	Functionalization for targeted delivery, enhanced stability and biocompatibility, potential for photothermal therapy
Zinc oxide nanoparticles (ZnO NPs)	Disruption of microbial cell membranes, inhibition of microbial enzyme activity and induction of oxidative stress	Photocatalytic activity against microbes, broad-spectrum antimicrobial activity, biocompatible and environmentally friendly
Titanium dioxide nanoparticles (TiO_2 NPs)	Photocatalytic generation of reactive oxygen species (ROS), disruption of microbial cell membranes	Photodynamic antimicrobial therapy, stability under various environmental conditions
Polymeric nanoparticles	Encapsulation and controlled release of antimicrobial agents, targeted delivery to specific sites, protection of antimicrobial agents from degradation	Versatile design for diverse applications, biodegradable and biocompatible

Targeted Delivery of Immunomodulatory Agents

Targeted delivery of immunomodulatory agents using nanoparticles represents a promising approach in immunotherapy, allowing for precise modulation of immune responses while minimizing off-target effects [277]. Nanoparticles offer several advantages as carriers for immunomodulatory agents, including their small size, high surface area-to-volume ratio, and tunable surface properties, which enable selective targeting of specific immune cell populations. Encapsulation of immunomodulatory agents within biocompatible and biodegradable nanoparticle formulations further enhances their stability, solubility, and pharmacokinetics, facilitating their delivery to target cells [278]. Here, we elaborate on this concept and provide examples of nanoparticle-based delivery systems for immunomodulatory agents:

Cytokines Cytokines play critical roles in regulating immune responses and can be utilized as immunomodulatory agents in the treatment of infectious diseases [279]. Nanoparticles can be engineered to deliver cytokines, such as interleukins and interferons, to specific immune cell populations. For example, polymeric nanoparticles loaded with interleukin-12 (IL-12) have been developed to selectively target dendritic cells and enhance antigen-specific T cell responses against infectious pathogens [280, 281]. Similarly, gold nanoparticles functionalized with tumor necrosis factor-alpha (TNF-α) have been shown to promote macrophage activation and enhance host defense against intracellular pathogens [282].

Antibodies Monoclonal antibodies (mAbs) can modulate immune responses by targeting specific cell surface receptors or signaling pathways. Nanoparticles can be conjugated with antibodies to achieve targeted delivery to immune cells expressing the corresponding antigen. For instance, lipid-based nanoparticles coated with anti-CD40 antibodies have been designed to selectively target dendritic cells and promote immune activation and antigen presentation [283]. Additionally, antibody-conjugated liposomes have been utilized to deliver immunomodulatory antibodies, such as anti-programmed cell death protein 1 (PD-1) or anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), to enhance antiviral immune responses and inhibit viral replication [284].

Small Molecules Small molecule immunomodulators, such as toll-like receptor agonists and immunosuppressive drugs, can be encapsulated within nanoparticle formulations for targeted delivery to immune cells. For example, polymeric nanoparticles loaded with toll-like receptor 7 (TLR7) agonists have been developed to activate innate immune responses and enhance antiviral immunity against respiratory viruses [285]. Similarly, lipid-based nanoparticles have been employed to deliver immunosuppressive drugs, such as dexamethasone or rapamycin, to suppress excessive inflammation and autoimmune responses while sparing non-target tissues [286].

Vaccine Delivery and Antigen Presentation

Vaccine delivery and antigen presentation have been revolutionized by the advent of nanoparticle-based vaccine platforms. These nanoparticles offer numerous advantages, including enhanced stability, controlled release kinetics, and improved immunogenicity, which have significantly advanced vaccine development efforts. Here, we elaborate on the features and cite examples of nanoparticle-based vaccine delivery systems:

Enhanced Stability and Controlled Release Kinetics Nanoparticles provide a stable and protective environment for antigens or antigenic peptides, shielding them from degradation and enzymatic cleavage. This enhanced stability ensures the integrity of the vaccine components during storage and transportation, preserving their immunogenicity until administration [287]. Furthermore, nanoparticles enable controlled release kinetics, allowing for sustained antigen presentation and prolonged immune stimulation. For instance, lipid-based nanoparticles have been engineered to encapsulate mRNA-based vaccines, ensuring their stability and controlled release for efficient antigen expression and immune activation [288].

Improved Immunogenicity Nanoparticle-based vaccines offer improved immunogenicity compared to traditional vaccine formulations, owing to their ability to enhance antigen uptake and presentation by antigen-presenting cells (APCs). Nanoparticles can be designed to mimic the size, shape, and surface properties of pathogens, facilitating their recognition and uptake by immune cells. Additionally, nanoparticles can be functionalized with immune-stimulating adjuvants, such as toll-like receptor agonists or cytokines, to enhance antigen presentation and immune activation [289]. For example, polymeric nanoparticles loaded with toll-like receptor agonists have been shown to enhance the immunogenicity of influenza vaccines, eliciting robust and long-lasting immune responses [290].

Targeted Delivery and Immune Activation Nanoparticles can be modified with targeting moieties or antibodies to enable precise delivery to immune cells expressing the corresponding receptors. This targeted delivery enhances antigen presentation and immune activation, leading to more potent immune responses against infectious agents [291]. For instance, gold nanoparticles conjugated with influenza virus antigens and mannose ligands have been shown to selectively target dendritic cells expressing mannose receptors, resulting in enhanced antigen presentation and T cell activation [292]. Additionally, lipid-based nanoparticles decorated with targeting ligands, such as antibodies or peptides, can efficiently deliver antigens to specific subsets of immune cells, promoting tailored immune responses against pathogens [293].

Immune-Modulating Adjuvants

The utilization of immune-modulating adjuvants delivered via nanoparticles represents a powerful strategy to enhance vaccine efficacy and promote robust immune responses against infectious pathogens. Here, we elaborate on this concept and provide examples of immune-modulating adjuvants delivered using nanoparticle-based vaccine formulations:

Activation of Innate Immune Pathways Nanoparticles can be loaded with immune-modulating adjuvants that activate innate immune pathways, such as Toll-like receptor (TLR) signaling, leading to the production of pro-inflammatory cytokines and chemokines. These adjuvants stimulate innate immune cells, such as dendritic cells and macrophages, to initiate and amplify immune responses against pathogens [294]. For instance, polymeric nanoparticles loaded with TLR agonists, such as CpG oligonucleotides or monophosphoryl lipid A (MPLA), have been shown to activate TLR signaling pathways and enhance antigen presentation, resulting in improved vaccine efficacy against viral and bacterial infections [295].

Controlled Release of Adjuvants Nanoparticles enables controlled release of immune-modulating adjuvants, prolonging immune stimulation and enhancing vaccine induced immune memory. By engineering nanoparticles to release adjuvants in a controlled manner, researchers can optimize the timing and duration of immune activation [296], leading to more potent and durable immune responses. For example, lipid-based nanoparticles loaded with adjuvants, such as alum or poly(I:C), have been designed to release adjuvants gradually over time, enhancing antigen-specific antibody and T cell responses against infectious pathogens [294].

Tailored Immune Responses Incorporating immune-modulating adjuvants into nanoparticle-based vaccine formulations allows researchers to tailor immune responses to specific pathogens and overcome immunological challenges associated with infectious diseases. By selecting adjuvants that stimulate desired immune pathways, researchers can enhance vaccine efficacy against intracellular pathogens, promote mucosal immunity, or induce long-lasting immune memory. For instance, lipid-based nanoparticles loaded with type I interferons (IFNs) have been developed to enhance antiviral immunity and protect against respiratory viral infections [297].

Immunomodulatory Nanotherapeutics

Immunomodulatory nanotherapeutics represents a promising approach for combating infectious diseases by precisely modulating immune responses using nanoparticle-based delivery systems. Here, we elaborate on this concept and provide examples of immunomodulatory nanotherapeutics:

Delivery of Immunosuppressive Agents Nanoparticles can be engineered to deliver immunosuppressive agents to mitigate excessive inflammation and tissue damage associated with severe infections [298]. For instance, polymeric nanoparticles loaded with corticosteroids, such as dexamethasone or prednisolone, have been developed to suppress immune responses and reduce inflammation in respiratory viral infections, such as influenza and COVID-19 [297, 299]. These nanoparticles enable targeted delivery of immunosuppressive drugs to inflamed tissues, minimizing systemic side effects and improving therapeutic outcomes.

Delivery of Immune-Stimulating Agents Conversely, nanoparticles can also deliver immune-stimulating agents to boost host immune responses and enhance pathogen clearance. For example, lipid-based nanoparticles loaded with toll-like receptor agonists, such as CpG oligonucleotides or poly(I:C), have been designed to activate innate immune pathways and promote antiviral immunity against respiratory viruses [300]. These nanoparticles enhance antigen presentation, cytokine production, and cytotoxic T cell responses, leading to improved viral clearance and reduced disease severity [301].

Dual-Function Nanoparticles Some nanoparticle-based therapeutics posses dual functions, combining both immunosuppressive and immune-stimulating properties to achieve therapeutic outcomes. For instance, mesoporous silica nanoparticles loaded with both anti-inflammatory drugs and immune-stimulating agents have been developed to modulate immune responses in autoimmune diseases, such as rheumatoid arthritis [302]. These nanoparticles deliver immunosuppressive drugs to suppress autoimmune reactions while simultaneously delivering immune-stimulating agents to enhance regulatory T cell responses and maintain immune tolerance.

4.8 Gene Therapy with Nanomedicine

Nanomedicine addresses several challenges associated with gene therapy, including delivery efficiency, off-target effects, and immune responses. Nanoparticles can be functionalized with targeting ligands, such as antibodies or peptides, to enhance specificity and uptake by target cells. Additionally, nanoparticle formulations can incorporate stimuli-responsive or biodegradable materials to achieve controlled release and minimize systemic toxicity [303]. Furthermore, nanomedicine enables the development of personalized gene therapies tailored to individual genetic profiles and disease characteristics, offering potential for precision medicine approaches in infectious disease management.

Enhanced Delivery Efficiency One of the primary challenges in gene therapy is achieving efficient delivery of therapeutic genes to target cells. Nanoparticles offer a solution by providing a protective and targeted delivery platform. For instance, lipid-based nanoparticles have been extensively utilized for delivering nucleic acids, such as mRNA or siRNA, to target cells with high efficiency. These nanoparticles protect the nucleic acids from degradation in the extracellular environment and facilitate their uptake by target cells through endocytosis mechanisms [304].

Minimization of Off-Target Effects Off-target effects, where therapeutic genes are delivered to unintended cells or tissues, pose a significant challenge in gene therapy. Nanoparticles can be functionalized with targeting ligands, such as antibodies or peptides, to enhance specificity and uptake by target cells while minimizing off-target effects [305]. For example, polymeric nanoparticles decorated with targeting ligands specific to cancer cells have been developed for delivering therapeutic genes selectively to tumor tissues, minimizing adverse effects on healthy tissues [306].

Reduction of Immune Responses Immune responses against viral vectors or therapeutic genes can limit the efficacy of gene therapy. Nanoparticle formulations can be designed to minimize immune responses by incorporating biocompatible and immunologically inert materials [307]. Additionally, nanoparticles can shield the therapeutic genes from immune recognition by encapsulating them within protective layers. For instance, gold nanoparticles coated with polyethylene glycol (PEG) have been shown to reduce immune responses and prolong the circulation time of nucleic acid payloads in vivo [308].

Controlled Release and Minimized Toxicity Nanoparticle formulations can incorporate stimuli-responsive or biodegradable materials to achieve controlled release of therapeutic genes and minimize systemic toxicity. For example, mesoporous silica nanoparticles can be engineered to release therapeutic genes in response to specific stimuli, such as pH or enzymes present in the target tissue microenvironment, thereby achieving precise control over gene delivery and minimizing off-target effects [309].

Precision Medicine Approaches Nanomedicine enables the development of personalized gene therapies tailored to individual genetic profiles and disease characteristics. By leveraging advances in genomics and nanotechnology, researchers can design nanoparticles that deliver therapeutic genes to specific cell types or tissues based on their genetic signatures [310]. This precision medicine approach holds promise for optimizing treatment outcomes and minimizing adverse effects in infectious disease management [311]. By harnessing the capabilities of nanoparticles, researchers can develop targeted and personalized gene therapies for infectious diseases, advancing precision medicine approaches in healthcare.

4.9 Combination Therapy Approaches

Combination therapy, involving the simultaneous or sequential administration of multiple therapeutic agents, has emerged as a promising strategy for effectively combating infectious diseases. In the nanomedicine era, combination therapy approaches are being increasingly explored to enhance treatment efficacy, overcome drug resistance, and minimize adverse effects [312]. This chapter delves into the rationale behind combination therapy in infectious disease management and discusses various combination therapy strategies facilitated by nanomedicine.

Rationale for Combination Therapy Infectious diseases often present complex challenges, including the emergence of drug-resistant pathogens, diverse mechanisms of pathogenesis, and the need for broad-spectrum antiviral [313] or antibacterial activity [314]. Combination therapy addresses these challenges by targeting multiple aspects of the infectious process, such as inhibiting viral replication, suppressing inflammation, enhancing host immune responses, and preventing microbial resistance. Additionally, combining therapeutics with complementary mechanisms of action can synergistically enhance treatment efficacy while reducing the risk of treatment failure due to single-agent resistance [315].

Synergistic Drug Combinations Nanomedicine enables the formulation and delivery of synergistic drug combinations with enhanced bioavailability, stability, and targeted delivery. For example, lipid-based nanoparticles can encapsulate multiple antiviral or antibacterial drugs, allowing for co-delivery to target sites with controlled release kinetics [316]. This approach maximizes drug synergy while minimizing systemic toxicity and adverse effects. Moreover, nanocarriers can be engineered to release therapeutic agents in response to specific stimuli, such as pH or enzymes present in the infected tissue microenvironment, further enhancing treatment specificity and efficacy [14]. Nanoparticle-based drug delivery systems offer improved bioavailability and stability for synergistic drug combinations. By encapsulating multiple drugs within lipid-based nanoparticles, such as liposomes or lipid nanoparticles, the drugs are shielded from enzymatic degradation and rapid clearance, leading to prolonged circulation times and increased drug concentrations at the target site. This enhanced bioavailability ensures effective delivery of therapeutic agents to the infected tissues, enhancing treatment efficacy [317]. Nanoparticles enable targeted delivery of synergistic drug combinations to the site of infection while minimizing systemic toxicity. Functionalization of nanoparticles with targeting ligands, such as antibodies or peptides, allows for specific recognition and binding to receptors overexpressed on infected cells or within the microbial niche. This targeted delivery approach enhances the accumulation of therapeutic agents at the infection site, optimizing treatment outcomes and reducing off-target effects [318]. Lipid-based nanoparticles have been extensively investigated for delivering synergistic antiviral drug combinations. For example, liposomes loaded with a combination of antiretroviral drugs, such as protease inhibitors and reverse transcriptase inhibitors, have been developed for the treatment of HIV/AIDS. These liposomal formulations offer enhanced stability and bioavailability of the drugs, leading to improved antiviral efficacy and reduced viral load in preclinical and clinical studies [319]. Polymeric nanocarriers loaded with multiple antibiotics, such as beta-lactams and fluoroquinolones, have demonstrated superior antibacterial activity against drug-resistant pathogens in vitro and in vivo. The controlled release of antibiotics from polymeric nanoparticles ensures sustained exposure to bacterial pathogens, leading to enhanced bacterial eradication and reduced emergence of resistance [320].

Combination of Antiviral Agents In the context of viral infections, combination therapy can involve the simultaneous or sequential administration of multiple antiviral agents targeting different stages of the viral life cycle [313]. For instance, nanomedicine facilitates the co-delivery of nucleoside analogs, protease inhibitors, and entry inhibitors to inhibit viral replication, maturation, and entry, respectively. By targeting multiple viral targets, combination antiviral therapy can prevent the emergence of drug-resistant variants and achieve more potent and sustained viral suppression. Viral replication involves multiple stages, including viral entry into host cells, replication of viral genetic material, assembly of new viral particles, and release of mature virions. Each stage presents potential targets for antiviral intervention [321]. Combination antiviral therapy aims to inhibit viral replication by simultaneously or sequentially targeting different stages of the viral life cycle. For instance, nucleoside analogs inhibit viral nucleic acid synthesis, protease inhibitors block viral protein processing, and entry inhibitors prevent viral attachment or fusion with host cells. Nanoparticles, such as liposomes, polymeric nanoparticles, or dendrimers, can encapsulate nucleoside analogs, protease inhibitors, and entry inhibitors within their matrix. These nanoparticles protect the antiviral agents from degradation, enhance their solubility, and enable their simultaneous delivery to target cells or tissues. By encapsulating multiple antiviral agents in a single nanoparticle formulation, nanomedicine facilitates combination therapy regimens that effectively target multiple stages of the viral life cycle [322]. Combination antiviral therapy enabled by nanomedicine offers a promising approach for effectively managing viral infections by simultaneously targeting multiple stages of the viral life cycle. By co-delivering antiviral agents via nanoparticles, nanomedicine enhances treatment efficacy, prevents the emergence of drug-resistant variants, and improves patient outcomes in the fight against viral diseases.

Integration of Immunomodulatory Agents Nanomedicine enables the combination of antiviral or antibacterial agents with immunomodulatory agents to enhance host immune responses and promote pathogen clearance. For example, lipid-based nanoparticles can deliver antiviral drugs along with immune-stimulating cytokines or toll-like receptor agonists to augment innate and adaptive immune responses against viral infections. This approach not only suppresses viral replication but also accelerates viral clearance and reduces the risk of reinfection [323]. By integrating immunomodulatory agents with antiviral or antibacterial agents, nanomedicine amplifies the immune response, leading to more effective pathogen clearance and improved treatment outcomes. Lipid-based nanoparticles serve as efficient carriers for co-delivering antiviral or antibacterial agents with immunomodulatory agents to target cells or tissues. Liposomes, for example, can encapsulate both hydrophilic cytokines or TLR agonists and hydrophobic antiviral or antibacterial drugs within their lipid bilayers or aqueous compartments. This co-delivery approach ensures the simultaneous release of therapeutic agents at the infection site, enhancing immune activation and pathogen inhibition [324]. Liposomal formulations containing both interferons and antiviral drugs have shown enhanced antiviral efficacy in preclinical studies of viral hepatitis and respiratory viral infections. These lipid-based nanoparticles promote both innate and adaptive immune responses, leading to accelerated viral clearance and reduced disease severity [303]. Immunomodulatory agents delivered via nanomedicine can augment innate immune responses by activating pattern recognition receptors (PRRs), such as TLRs, on immune cells. TLR agonists, when co-delivered with antiviral drugs, stimulate the production of pro-inflammatory cytokines and type I interferons, which play crucial roles in orchestrating antiviral immune responses. This synergy between antiviral agents and immunomodulatory agents enhances viral clearance and reduces the risk of viral dissemination and reinfection [325].

Personalized Combination Therapy Advances in nanomedicine and precision medicine enable the development of personalized combination therapy regimens tailored to individual patient profiles, disease characteristics, and pathogen susceptibility patterns. By leveraging diagnostic tools, such as genomics, proteomics, and imaging modalities, clinicians can optimize treatment strategies and select the most effective combination of therapeutics for each patient [326]. This personalized approach maximizes treatment efficacy while minimizing adverse effects and treatment-related complications. Combination therapy approaches facilitated by nanomedicine offer promising strategies for effectively managing infectious diseases in the modern healthcare landscape. By combining synergistic drug combinations, integrating immunomodulatory agents, and adopting personalized treatment regimens, clinicians can optimize treatment outcomes, overcome therapeutic challenges, and improve patient care in the nanomedicine era. Table 12 provides an overview of combination therapies.

5 Challenges and Future Directions

While nanomedicine holds immense promise in revolutionizing the management of infectious diseases, several challenges must be addressed to fully harness its potential. Understanding and overcoming these challenges are crucial for navigating infectious challenges in the nanomedicine era.

Drug Resistance One of the major obstacles in combating infectious diseases is the increasing prevalence of drug-resistant pathogens. Despite advances in nanomedicine, pathogens can develop resistance to nanoparticle-based antimicrobial agents, leading to treatment failure [327]. Future research efforts should focus on developing strategies to mitigate and overcome drug resistance, such as the rational design of nanoparticles targeting multiple microbial vulnerabilities and combination therapies that synergistically combat resistant strains.

Biocompatibility and Safety Ensuring the biocompatibility and safety of nanomedicines is essential for their clinical translation. Nanoparticles may trigger immune responses, cause cytotoxicity, or accumulate in off-target tissues, posing risks to patient health. Addressing these safety concerns requires comprehensive preclinical evaluation of nanomedicine formulations, including assessment of their pharmacokinetics, biodistribution, and long-term toxicity profiles [328]. Furthermore, the development of biodegradable and non-toxic nanomaterials is crucial for enhancing the safety profile of nanomedicines.

Optimal Targeting and Delivery Achieving precise targeting and controlled delivery of nanomedicines to infection sites remains a challenge. Factors such as nanoparticle size, surface charge, and surface functionalization influence their pharmacokinetics and biodistribution [329]. Additionally, the heterogeneous nature of infectious lesions, such as biofilms or intracellular pathogens, presents obstacles to effective nanoparticle delivery. Future research should focus on optimizing nanoparticle properties and engineering strategies to enhance their targeting specificity and penetration into infection sites.

Clinical Translation and Regulatory Hurdles The clinical translation of nanomedicines faces regulatory hurdles and challenges related to scalability, manufacturing consistency, and cost-effectiveness. Regulatory agencies require robust preclinical and clinical data to evaluate the safety and efficacy of nanomedicine formulations [330]. Furthermore, the high cost of nanoparticle synthesis and scale-up process presents barriers to widespread clinical adoption. Collaborative efforts between academia, industry, and regulatory agencies are essential for advancing nanomedicine from bench to bedside and addressing regulatory challenges.

Future directions

Future directions in the field of nanomedicine for infectious diseases encompass precision medicine approaches, multifunctional nanotherapeutics, and

Table 12 Overview of combination therapies used in nanomedicine era

Disease	Mechanism of action	Combination therapies
Cancer	Targeting multiple signaling pathways, apoptosis induction, DNA damage repair inhibition.	Combination of chemotherapy agents, targeted therapies, immunotherapies, and gene therapies.
HIV/AIDS	Inhibition of viral replication, maturation, and entry.	Combination antiretroviral therapy (cART) comprising nucleoside analogs, protease inhibitors, and entry inhibitors.
Bacterial infections	Inhibition of bacterial cell wall synthesis, protein synthesis, nucleic acid synthesis.	Combination of antibiotics targeting different bacterial pathways (e.g., cell wall and protein synthesis).
Inflammatory diseases	Suppression of inflammatory cytokines, immune cell activation.	Combination of anti-inflammatory drugs, disease-modifying antirheumatic drugs (DMARDs), and biologic agents.
Cardiovascular diseases	Reduction of blood pressure, cholesterol levels, thrombosis prevention.	Combination of antihypertensive agents, lipid-lowering drugs, antiplatelet agents, and anticoagulants.

nanotechnology-enabled diagnostics. Precision medicine holds the potential to revolutionize infectious disease treatment by tailoring therapies to individual patient characteristics and pathogen profiles. By integrating nanomedicine with precision medicine, personalized treatments can be developed based on factors such as genetic predisposition, immune status, and microbial susceptibility patterns. This approach not only optimizes treatment outcomes but also minimizes adverse effects by delivering targeted therapies. Additionally, the development of multifunctional nanotherapeutics represents a promising avenue for combating infectious diseases. These nanomedicines can simultaneously target multiple aspects of infection, combining antimicrobial agents with immunomodulators, diagnostic imaging agents, or therapeutic payloads. Such multifunctional approaches enable synergistic therapeutic effects and real-time monitoring of treatment responses, enhancing treatment efficacy and patient outcomes. Furthermore, nanotechnology-enabled diagnostics offer innovative solutions for the rapid and sensitive detection of infectious pathogens. Nanoscale biosensors, nanoparticles-based imaging probes, and point-of-care diagnostic devices can facilitate early diagnosis and treatment initiation, enabling precision-guided therapies and effective outbreak containment strategies. Overall, these future directions underscore the transformative potential of nanomedicine in revolutionizing the diagnosis, treatment, and prevention of infectious diseases.

6 Ethical Consideration

Ethical considerations are foundational pillars in the realm of nanomedicine for infectious diseases, permeating every facet of research, development, and implementation. At the forefront lies the principle of equitable access, advocating for the fair distribution of nanomedicine interventions irrespective of socioeconomic status or geographic location [331]. This necessitates concerted efforts to bridge healthcare disparities and ensure that vulnerable populations have access to these innovative technologies. Central to ethical practice in nanomedicine is the principle of informed consent, wherein patients are provided comprehensive information about the nature, risks, and benefits of nanomedicine interventions, empowering them to make autonomous decisions about their healthcare journey [332]. Privacy and data security emerge as critical concerns in the era of nanomedicine, demanding robust measures to safeguard sensitive health information against unauthorized access or misuse. Transparency and accountability underscore ethical conduct, necessitating open communication, disclosure of potential conflicts of interest, and adherence to regulatory standards. Moreover, ethical decision-making entails a thorough risk-benefit assessment, weighing the potential advantages of nanomedicine against its associated risks and uncertainties to prioritize patient welfare. By upholding these ethical principles, researchers and healthcare providers can navigate the complexities of nanomedicine ethically and responsibly, fostering trust, equity, and patient-centered care in the fight against infectious diseases.

7 Conclusion

This book chapter on nanomedicine presents a detailed exploration of the field's vast landscape, starting with an insightful introduction to its foundational concepts. Delving into nanoparticles and drug delivery mechanisms, the discussion illuminates their intricate role in enhancing therapeutic efficacy. The chapter further delves into the realm of diagnostic nanotechnology, offering a nuanced analysis of nanosensors for pathogen detection, the transformative potential of nanoparticles in imaging techniques, and the development of cutting-edge nanoparticle-based biomarkers and molecular diagnostics. Transitioning seamlessly into therapeutic applications, the chapter navigates through a diverse array of interventions, from nanoantibiotics and antiviral nanoparticle to innovative nanovaccines and intracellular drug delivery systems. Addressing challenges head-on, such as drug resistance and ethical considerations, the chapter advocates for responsible use and ethical frameworks in nanomedicine. Looking towards the future, it outlines emerging trends and forward-thinking approaches, including the burgeoning field of gene therapy within nanomedicine and the promising avenues of combination therapy strategies. Through a holistic lens, this chapter encapsulates the dynamic landscape of nanomedicine, highlighting its transformative potential and shaping the trajectory of healthcare advancements.

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Defining Nanomedicine Qualities for Optimal Therapeutic Applications

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1 Introduction

The term "nanomedicine" (Greek for dwarf is *nanos*) [1] is an application of nanotechnology in healthcare. Nanomedicine is a new interdisciplinary field of research that deals with the application of nanotechnology in health and medicine for the prevention, diagnosis and treatment of diseases. The use of nanoparticles in therapy is on the verge of revolutionization, although only a few nanoparticle-based formulations have reached the market for safe and effective clinical translation [2]. Nanomedicine involves the development of nanostructured surfaces and nanoparticles which have the potential to transform disease diagnosis, treatment, and monitoring at the molecular and cellular levels. It is an emerging field, that works on a billionth of a meter scale, exploiting the unique properties of the nanomaterials, which are helpful for specialized drug delivery systems, upgrading imaging techniques, and paving the way for personalized medication.

When compared to their bulk counterparts, these nanoparticles have significantly higher surface area to volume ratios [3] increasing the interaction ability with biological systems and in the transport of therapeutic cargoes. These particles have unique physicochemical features that may be modified to enhance their interactions with biological systems. There are many applications of nanomaterials including medical imaging, drug delivery, photothermal therapy, and magnetic hyperthermia

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[4]. These properties can be leveraged to improve bolster immune regulation and enhance drug delivery, and tumor targeting, tackling some primary challenges in tumor immunotherapy [5, 6]. The site and size-specific properties of nanomedicine have the potential to improve the therapeutic index, reduce the incidence and intensity of side effects that are commonly associated with traditional drug delivery methods [7] and thereby resulting in improved patient acceptance, compliance and prognosis [8].

With these nanomedicines, cytotoxic drugs are specifically delivered to the areas which increase their concentration in the targeted tissue and reduces damage to non-targeted areas with minimum side effects and more therapeutic outcomes at low cost [9]. Nanoparticles can be specifically made to act as contrast agents, to improve the sensitivity and resolution of medical imaging techniques such as computed tomography (CT) scans and magnetic resonance imaging (MRI) [10]. These nanoparticles can detect the abnormalities at an early stage, allowing for more rapid and effective treatment. Nanoparticles can detect the tumor by illuminating the cancerous cell in an MRI scan by binding to it [11]. Thus, exciting horizons are being explored by investigators concerning theranostic, for example, where a single nanoprobe can serve two functions: diagnosis and treatment [12]. Additionally, nanotechnology can be used in the field of tissue engineering [13]. Researchers are trying to design scaffolds for tissue engineering. These scaffolds are made up of biocompatible nanoparticles having special properties that can help regenerate damaged tissues by creating scaffolds using nanomaterials, for patients suffering from debilitating conditions [14].

However, as with any revolutionary project, nanomedicine faces substantial obstacles. To study the long-term effects of nanoparticles on human health and as well as on the environment is extremely important [15]. It is important for these nanoparticles to be biocompatible and biodegradable, as unexpected consequences might dominate the potential benefits. Stringent rules and safety methods are vital to ensure safe and feasible practice [16]. Further research and development are instrumental for this innovative field to reach its full potential.

2 Classification

Nanomedicine can be classified into two types based on the composition of the nanomaterials used– organic and inorganic nanomedicine. Organic nanomedicines are typically composed of polymers or lipids, whereas inorganic nanocarriers contain metals, ceramics, or quantum dots. Inorganic nanomedicine involves man-made nanoparticles such as gold, silver or iron oxide, which are less than 100 nm [17, 18] (Fig. 1). Each material has unique qualities that have an impact on the nanocarrier's suitability for a given application [19]. Nanomedicines can also be categorized according to their size, shape, function (drug delivery, bioimaging, theragnostics, etc.), targeting strategy (passive or active), mode of administration, and interactions with the biological system (immune responses, clearance pathways, tissue regeneration, etc.) [20, 21].

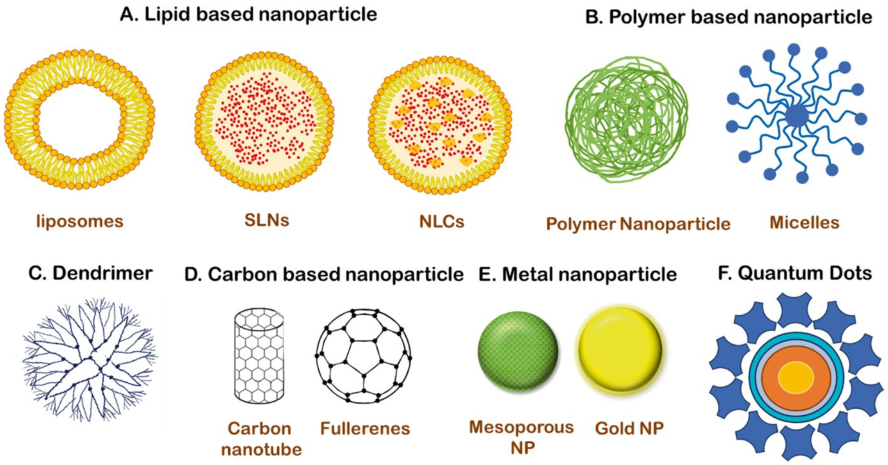

A. Lipid based nanoparticle
B. Polymer based nanoparticle
liposomes
SLNs
NLCs
Polymer Nanoparticle
Micelles
C. Dendrimer
D. Carbon based nanoparticle
E. Metal nanoparticle
F. Quantum Dots
Carbon nanotube
Fullerenes
Mesoporous NP
Gold NP

Fig. 1 Classification of nanomedicines. (**a**) Lipid based nanoparticles: Liposomes, Solid Lipid Nanoparticle (SLN), Nano Lipid Carriers (NLCs), (**b**) Polymer based nanoparticle: Polymer nanoparticle, Micelles (**c**) Dendrimer (**d**) Carbon based nanoparticle: Fullerenes, Carbon nanotubes, (**e**) Metal nanoparticle: Mesoporous and Gold nanoparticle, (**f**) Quantum Dots

2.1 Organic Nanomedicines

Organic nanomedicine is revolutionizing the health industry, as it is premised on naturally existing or synthetic organic molecules that can be engineered to produce nanomedicine for therapy and diagnosis (Fig. 1). Polyethylene glycol or the natural chitosan can be used as drug carriers, increasing there solubility in the body and controlling the release at a specific rate. Liposomes are biocompatible spheres made up of fatty molecules that can be used to deliver hydrophobic drugs directly to a targeted cell. Thus, the benefits of organic nanomedicine are that it is biocompatible, organic-based, composed of vast depositories, affording a specially tailored scheme for different applications, and with a low potential for side effects.

Additionally, synthetic polymers provide the capacity to encapsulate drugs in small spheres, control the rate of release, and enhance a drug's water solubility [22]. However, natural polymers are biodegradable and biocompatible so that long-term toxicity is reduced [23]. Liposomes are small balls, full of fatty molecules that serve as safe space for hydrophobic drugs. Thus, targeting ligands for more accurate delivery of a drug is possible [24]. Organic nanomedicine has three main advantages: biocompatibility results in fewer adverse reactions, versatility is good for accurately modified design structures to align with certain needs, and delivery improvement ensures that a controlled release mechanism is maintained [25].

Lipid Based Nanoparticles

Lipid-based nanoparticles are one of the most powerful drug delivery agents in the realm of organic nanomedicines. Microscopic carriers for the delivery are constructed from natural or synthetic lipids which provide a new and innovative approach for administering, targeting, and delivering medicine into cells of interest. Each type of nanoparticle offers unique strengths for various conditions. Liposomes represent various degrees of similarity to cellular membranes and are an excellent choice for drug delivery [24]. Solid Lipid Nanoparticles (SLNs) are efficient in coating hydrophobic drugs, thus providing a long-term effect. Nanostructured Lipid Carriers (NLCs) have greater nanomedicine potential, as it combines the best plasticity of the loading and coating mechanism, with an efficient release. There are a wide range of benefits of lipid based nanoparticles. First, biocompatibility helps to prevent side effects. Secondly, these nanoparticles could be designed to store a large array of drugs. Furthermore, surface modifications guarantee targeted delivery, thus escorting drugs to diseased cells while limiting exposure to normal tissues. Additionally, controlled-release mechanisms enhance drug delivery [26].

Liposomes

Liposomes are lipid-based nanocarriers that are the forerunners of all drug delivery options [27]. These circular spheres are made up of natural or synthetic phospholipids [28]. Liposomes, commonly noted for their amphiphilic characteristics, acquire comparable charm to cell membranes due to this property [29]. It implies they can conveniently interact with all cells and thus, become a valuable vehicle for transmitting the drug. In other words, they may hide hydrophobic drugs in lipolayers and dissolve them in hollow cores in water [30]. Liposomes are composed of concentric bilayers of phospholipids that produce spherical vesicles, the diameter of these spheres may vary from 50 to 500 nm [31]. Imagining phospholipids as small balls, the head of this ball attracts water, on the other hand, its tail is averse to water. They create dual layers with tails hidden inside the membrane and heads exposed. The core of this hollow sphere has adequate area to hold many therapeutic agents [24].

There are several species of liposomes. Traditional liposomes are the simplest, but they lack good control over drug release as a consequence, and their therapeutic use is limited [32, 33]. Long-circulating liposomes are a variant of the first species that have been chemically modified with the Polyethylene Glycol (PEG) polymer to stay in the bloodstream for a longer time, and thus, can efficiently reach their target site [34]. Targeted liposomes use special molecules on their surface, much like guided missiles [35]. It distinguishes them from others as they ‘know’ which cells they need to act on and which ones to avoid and consequently have fewer side effects [36]. At last, stimuli-responsive liposomes act as tiny vaults. However, unlike cannonballs, they do not open until they leave the bloodstream which opens under certain conditions, such as a change in pH or temperature. This process allows for the drug to be released precisely on the target site [37]. Liposomes can be used as carriers of many drugs, particularly hydrophobic drugs. They can also be used to transfer genetic material for gene therapy [38] or as adjuvants in vaccines to stimulate an immune response [39]. Furthermore, liposomes containing imaging agents can improve medical imaging techniques.

Therefore, the advantages of liposomes are many. First, the phospholipids, components of cellular membranes, are harmless. Second, liposomes have versatility, as substances of various nature may be loaded into them and delivered to the optimal place [40]. Finally, they can be navigated to target cells, which may significantly decrease extraneous toxic side effects [41]. At the same time, there is a list of difficulties associated with these. Conventional liposomes are unstable and can fuse with one another reducing the surface tension [42], and optimized formulations are expensive, so mass production needs to be established. Despite all these problems, there are enough prospects for how research can evolve.

With growing knowledge of the characteristics of nanoparticles, the stability of classic liposomes will likely no longer be a restriction. Having developed a new approach, targeting offers the ability to partially close the gap between promising *in vitro* targeting and actual *in vivo* usage. Also, liposomes are novel co-delivering techniques that can change the way combination therapy is implemented thus increasing the treatment efficacy in breast cancer cells by encapsulation of two drugs resveratrol and paclitaxel [43]. Finally, new targeted strategies may contribute to directed therapy, which has the potential for developing personalized medicine.

Solid Lipid Nanoparticles

Solid Lipid nanoparticles (SLNs) represent a novel generation of lipid drug carriers that have the potential to revolutionize the administration of medication [44]. Unlike liposomes, which have a lipid bilayer organization, SLNs have a solid core of lipids solely containing drug molecules and are surrounded by a thin layer of protective material. This novel organization has several advantages. First, the solid core guarantees maximum stability [45]. SLNs are more impervious to decomposition during storage and transit facilities than liposomes, making them a significantly better alternative. Second, the solid matrix allows for the packaging of more medication which is helpful for drugs that are poorly soluble in water [46].

SLNs can be produced from natural lipids, including triglycerides and steroids such as cholesterol, and waxes [45]. Each of them has certain advantages. For example, triglycerides are well handled by the body and promote controlled drug release. Because they are derived from natural lipids such as liposomes, they are usually more biocompatible and have fewer adverse effects. They are excellent for accommodating poorly water-soluble medications; they help to control the release rate possessing the potential to modulate the medication dose simply by changing the composition or the structure of the solid core [47]. Concerning liposomes, SLNs main advantage is their superior stability which makes them ideal for long-term storage and transport [46].

One of the disadvantages of SLN is that though the drug loading ability of SLNs is higher than that of liposomes, some SLNs may not be able to encapsulate enough drugs [46]. In addition, the solid core can crystallize during storage or fluctuations in temperature, influencing drug release [48].

Nanostructured Lipid Carriers

Nanostructured Lipid Carriers (NLCs) are currently the “next generation” of lipid-based drug delivery systems. NLCs combine the best of Solid Lipid Nanoparticles and traditional liposomes [49]. NLCs have solid lipid cores similar to SLNs. Due to this factor, the NLCs demonstrate similar stability, controlled release, and high drug encapsulation capacity [50]. The core of SLN is an ordered crystalline and has a high drug load, while the NLCs contain liquid oil in the crystalline matrix of hard lipids, giving it a less ordered, “imperfectly” crystalline structure. It is this minute difference that gives an advantage in the form of space [51]. That is why NLCs can contain much more of the drug than any other carrier, including conventional SLNs.

NLCs have a variety of advantages. They are flexible since they are compatible with a wide range of drugs, both hydrophilic and hydrophobic [52]. The ratio of solid lipid to liquid oil can be changed to optimize drug loading and release rates. They combine the stability advantages of SLNs and facilitate long-term storage and transportation. The drug-loaded nanocarriers are cost-efficient to produce and employ in high-scale clinical applications [53]. The formulation process of such carriers may be difficult to optimize, with the necessity to cautiously choose lipid components and drug loading parameters. When combined with targeting antibodies or ligands, NLCs can make a significant contribution to delivery [50]. The delivery of two or more medications at the same time is achievable due to combination therapies [54]. Hence, NLC can be used as personalized medicine.

Polymer Based Nanoparticles

Polymer-based nanoparticles and micelles are synthetic, derived from a wide variety of polymers, and provide an effective means for drug delivery. They can envelope multiple types of medications and transport them directly to their intended targets. Polymer-based nanoparticles are minute molecules bound by natural or synthetic polymers. The polymer determines the smallness, surface charge, and release rate of their cargo enabling these particles to be tailored to specific drugs and delivery requirements [55]. Furthermore, they may have molecules on their surface, ensuring that only a small quantity of the prescribed medication is present in the remainder of the body, minimizing any preconditions [56]. Polymer-based nanoparticles may be used as anticancer medications as well as gene therapy agents [57]. Furthermore, they may be filled with imaging agents beforehand to assist with medical imaging [58–60]. Different forms of these particles are present; each has its own unique set of benefits. Polylactic-co-glycolic acid (PLGA) nanoparticles are some of the most widely researched polymer-based nanoparticles because they are biodegradable and biocompatible. They can be utilized to transport a variety of medications, including anticancer drugs, vaccines, and gene therapy agents [61]. Chitosan nanoparticles used in natural polymers have mucoadhesive characteristics, allowing them to adhere to mucus constituents, which makes them useful for delivering medicines to the lungs and alimentary canal [62]. PEGylated nanoparticles use PEG on their surface, increasing the nanoparticles' water solubility, to evade the immune system for more circulation in the body [63, 64], and can be further conjugated with targeted ligands for the targeted drug delivery [65].

Micelles

Micelles are spheres that spontaneously assemble due to amphiphilic polymers [66]. Micelles are molecules that are created from polymers consisting of a core encased in a shell [67]. These tiny spheres carry poorly soluble (hydrophobic) drugs to the target area, revolutionizing the treatment of many diseases [68]. The amphiphilic polymers' water-hating tails cluster inside, forming a core that surrounds hydrophobic drugs. Meanwhile, the hydrophilic heads push out to keep the micelle afloat in water [69]. Micelles have several features that make them beneficial when treating patients. The hydrophobic core acts as a protective environment, allowing the drug to better disperse throughout the body and increase its biocompatibility [70]. These minuscule spheres aid in transporting drugs with solubility to regions leading to progress in the treatment of diseases [68]. Essentially micelles are structures that come together naturally because of types of polymers that have both water attracting and water repelling properties [66]. In addition, their small size, which ranges from 10 to 100 nanometres, enables them to accumulate passively in tumors, a phenomenon known as enhanced permeability and retention (EPR). The EPR allows micelles to be more abundant in tumor tissue than in most tissues [71]. Due to the leaky nature of cancer tissues, micelles are easily infiltrated [72]. Micelles have other distinct benefits, including their diversity. By combining multiple amphiphilic polymers, researchers might develop micelles with various properties suitable for other medications and drugs.

Dendrimers

Dendrimers are one of the most useful nanomedicines. These are highly branched multi-layered structures similar to trees which have various advantages. As these are branched structures it provides space for the packaging of drugs. This allows more effective drug delivery and has a release rate that can be controlled upon design [73]. Moreover, dendrimers have an exterior that is ligated using targeting ligands that limit them to specific cell activity, sparing neighbouring healthy tissue [74]. They are preferable to use for the delivery of several types of drugs like cancer therapy agents, gene therapy agents, or antibiotics [75] and can load imaging agents for scans [76] or function as sensors [77] and catalysts [78].

Dendrimers act as delivery agents for the delivery of poorly soluble anticancer drugs. Polyamidoamine (PAMAM) dendrimers are studied for their high delivery capacity of the anticancer drug paclitaxel [79]. In addition, dendrimers contribute to gene therapy. They can be a variety of non-viral vectors, or vehicles that safely deliver genetic material for gene therapy. This capability arises from their ability to form complexes with DNA and protect it from degradation [80]. Also, dendrimers are combined with antibiotics which in turn, increases antibiotic effectiveness. They can also be used to fight against antibiotic resistant bacteria by increasing antibiotic solubility. Overall, the drug is targeted to areas of infection, making dendrimers a potential weapon against antibiotic-resistant bacteria [81]. They are also used in diagnostics. Dendrimers are connected with gadolinium which is the most used contrasting agent in MRI. Dendrimer-gadolinium is a good conductor of contrasting MRI, which makes it possible to improve diagnostic capabilities for diseases [82].

Carbon-Based Nanoparticles

Carbon-based nanoparticles represent a unique and exciting class of materials for use in nanomedicine, enabled largely by their intriguing structures. They serve as a platform for targeted drug delivery, increased drug performance, and new therapeutic possibilities. There are several applications of these molecules in therapeutics [83]. Fullerenes are the most known carbon-based nanoparticles, which look like soccer balls [84]. These spheroidal arrangements of sixty carbon molecules are fitted together to form a closed cage of pentagons and hexagons, making them excellent fillers for drugs [85]. The source and organization of these carbon atoms influence properties such as electrical conductivity and reactivity [86]. Carbon nanotubes, which are available in single and multi-walled forms, are one million times stronger than steel and conductors [87]. Graphene, known as the “wonder material”, consists of a layer of carbon atoms arranged in a hexagonal lattice structure. This extraordinary two-dimensional substance exhibits conductivity, mechanical stability, and transparency [88].

Efforts are being made by scientists for the construction of highly compatible, and degradable carbon nanostructures that would increase the efficiency of targeted therapeutic agents. Researchers are working to enhance surface modification methods to elevate effective targeting abilities with controlled drug release. Carbon-based nanoparticles are the future of medicines, and it will be a new age of treatments. Therefore, scientists are working on designing more biocompatible and degradable carbon nanostructures with increasing drug loading capacity of nanoparticles to aid in the development of targeted therapeutic agents.

2.2 *Inorganic Nanoparticles*

The utility of inorganic nanoparticles inside the field of nanomedicine is revolutionizing the biomedical field. Inorganic nanoparticles' main advantage is their ability to alter their size, shape, and surface biochemistry, which tailors them to act on certain pathological cells while being minimally dangerous to healthy tissues [89]. Moreover, some inorganic nanoparticles when considering the instance of gold-based nanoparticles have unfavorable biocompatibility and are entirely harmless [90, 91]. In addition, the inorganic nanoparticles have other intriguing features. They can be engineered to function as a drug deliverer, an imaging agent, and a therapeutic agent [89]. In addition to the broad range of therapeutic applications, inorganic nanoparticles are not an exception. Two of the most typical examples include metal nanoparticles, appearing for gold or iron oxide, as well as quantum dots [18].

Metal Nanoparticles

Metals as nanoparticles represent some of the most promising and significant tools in nanotechnology, especially they can be used in diagnosing and treating diseases. The diameter of metal NPs (MNPs) ranges between 1 and 100 nm. These metal NPs have exceptional catalytic, antimicrobial and optical properties and targeted drug delivery in cancers. Thus, they serve as great potential in the field of nanomedicine. They have distinctive general properties like shape, size, colour, electronic surface charge, surface density, reactivity, sensitivity, and surface-volume ratio. Nearly all metals can be converted to NPs. The most used metals to synthesize nanoparticles are iron, aluminum, silver, gold, copper, zinc, cadmium, and cobalt [92].

MNPs' specific structure opens up quite a few nanomedicine opportunities [93]. Specific MNPs can be used as contrast agents in medical imaging procedures to allow improved visualization of tissue or organ [94, 95]. Moreover, MNPs seem to be an exciting option for hyperthermia therapy, which involves killing cancer cells by heating them [96]. Silver nanoparticles have intrinsic antimicrobial effects, which means they might be used to cure illnesses caused by bacteria or fungi [97].

There are many types of MNPs currently being researched for medical use. The best-known biocompatible particles are gold nanoparticles that have been dyed for drug delivery, tumor heating photothermally, or a contrast agent for imaging [98]. Mesoporous silica nanoparticles are not metal but are made of metal-containing elements and are used for drug delivery and imaging for easy adjustment by a large area and pore [99].

Quantum Dots

Typically, the size of Quantum Dots (QDs) ranges between 2–10 nm in diameter [100]. QDs are considered as a promising tool for applications like drug delivery [101], diagnostics, and medical imaging [102]. They are used to depict cells in fluorescent form. They are called shining stars as they are known for their unique properties in the field of nanomedicine [103]. QDs have great potential which allows them to give fresh information and measurements of cells. QDs have an advantage over traditional organic dyes as they produce more stable and brighter light due to their exceptional fluorescence. They are preferred over natural fluorophores due to their properties like photostability, restricted emission, effective sensitivity, and single excitation source. Thus, QDs are able to generate highly sensitive image of biological targets. For example, QDs can be used to distinguish different biomolecules in distinct colours within a cell. QDs are used as a novel approach in drug delivery since their fluorescence can be used to track the distribution and uptake [104].

The use of photodynamic therapy (PDT) has increased during the past few decades, but due to the limitations of the traditional photosensitizers used, Nanotechnology-integrated PDT has recently been studied. The potential of QDs as PSs or PSs carriers, to enhance PDT, a light-based cancer treatment, is now being investigated. Reactive oxygen species (ROS) can be formed by either energy transfer (Type II) or electron transfer (Type I), leading to two different PDT processes. During the type II photodynamic reaction, excited PS molecules convert molecular oxygen into highly active singlet oxygen which upon interaction with macromolecules leads to death of the cells [105]. QDs hold promise for PDT due to their distinct properties. Scientists can manipulate the color of emitted light by precisely controlling the size of the QD. The preliminary results of QD as a photosensitizer in PDT have shown significant and promising results which guarantees further investigation in the development (Uprety and Abrahamse, 2022). Currently, there is very little information available on the pharmacodynamics and pharmacokinetics of QDs. There are reports which show long term toxicity of QDs on humans and the environment. Current research needs to discover modifications in QDs for safe use.

3 Therapeutic Nanomedicine

When compared to traditional pharmaceuticals, clinical outcomes for nanotherapeutics exhibit unique advantages. The last three decades have witnessed extensive research in nanomedicine, which has resulted in the global commercialization of many nanomedical products as well as the translation of channeled candidates. Since these characteristics give nanotherapeutics minimal toxicity, increased bioavailability, improved pharmacokinetics, and improved therapeutic effect, they frequently show higher therapeutic efficacy than traditional small molecular medicines. Hence, nanotherapeutics get over barriers that traditional medications cannot, and they may be able to satisfy market demands for illness treatments in the future [106]. Additionally, it is anticipated that the more than 400 nanomedicine formulations, presently undergoing clinical trials, will offer innovative clinical solutions (such as macromolecule delivery platforms) either by themselves or in conjunction with other critical enabling technologies, such as advanced biomaterials, microfluidics, indigenous systems, optical physics, automation, linens, data processing and digital technology [16]. Comprehensively, therapeutic nanomedicine is the epitome of evolution of healthcare, offering hitherto unexplored possibilities for more accurate, efficient, and safe illness assessment and therapy. There still exists a potential for innovative medical discoveries as long as research in this domain is conducted.

3.1 System and Strategies

Recently, there have been immense developments in the field of delivery systems to provide therapeutic agents to its target locations. Although many drug delivery systems have been effectively used recently, there are still some issues that need to be resolved. Research is currently being done on nano-based drug delivery systems, which will enable enhanced drug delivery [107]. These systems include liposomes, polymeric micelles, nanocrystals, dendrimers, polymer-drug conjugates, and carbon nanotubes [108]. Targeted drug delivery systems via nanocarriers offer many advantages over conventional chemotherapy as their specificity minimizes adverse effects on non-target tissue [109]. These targeting strategies include-

Passive Targeting

This approach is mostly applicable to cancer therapy. The main feature of tumors is their uncontrolled and rapid growth, due to which it require more oxygen and nutrients. Thus, new blood vessels are formed to provide the needed requirements [108]. Due to unbalanced pro-angiogenic factors, these blood vessels develop an abnormal and tortuous structure with discontinuous epithelium and a defective basement membrane. In the healthy tissues, the lymphatic system continuously removes and returns, solutes, extravasated water, and colloids from interstitial fluid back to circulation. But in the tumor tissue, the lymphatic drainage is generally poor and the dynamic transport of interstitial fluid is frequently disrupted. The molecules that are leaked to interstitial fluids of the tissue are held up for long time periods. This phenomenon is known as the "Retention" portion of the EPR effect [110, 111].

The efficiency of EPR-based drug targeting is critically determined by the physicochemical characteristics of macromolecules or nanoparticles, such as their surface features, size, shape, and biocompatibility. For instance, molecules greater than 200 nm are poor in tumour extravasation, while molecules smaller than 40 kDa (corresponding to nanoparticles with hydrodynamic diameters of 5–6 nm), the threshold size for renal clearance, typically have short lifespans in the circulation.

The nanoparticle with diameter 50–150 nm is considered as the ideal size for EPR directed tumor targeting [111].

Although EPR effect is accepted for developing tumor targeting nanomedicine, in past decades the heterogeneity of the EPR effect is increasingly recognized. This heterogeneity results from the complexities of tumor pathophysiology which include tumor type, size, location, stage, Tumor microenvironment, etiology, vascular density, and of blood perfusion status. To overcome this problem, many EPR augmenting methods can be employed which include the administration of vascular or inflammatory mediators like TNF-α, angiotensin-II, and bradykinin, and physical approaches like sonoporation, and hyperthermia, radiotherapy can be implemented to enhance EPR effect [111] (Fig. 2).

Active Targeting

Active targeting is an alternative approach used in delivering the drug as there are some limitations of passive targeting. For instance, in a bone tumor, where the irrigation of the tissue is indisposed, nanoparticles have significant difficulties reaching therein. Tumour masses are made up of cell types, including immunological, supporting, and healthy cells from the original tissue as well as tumor cells [110]. Therefore, to have an effective therapeutic impact, nanoparticles must be able to identify cancerous cells and target them with their action. This can be achieved by so-called active targeting. This can be done by attaching ligands like antibodies, aptamers, peptides, or small molecules that can only bind to the specific receptors located on the tumor cells. This approach will improve the affinities of the nanomedicines and enhance their penetration. To attain high specificity those receptors need to be substantially expressed on tumor cells but not on normal cells [108, 112].

Additionally, the receptors must be expressed uniformly and not leak into the bloodstream. First, targeting conjugates attach to their receptors, and then the

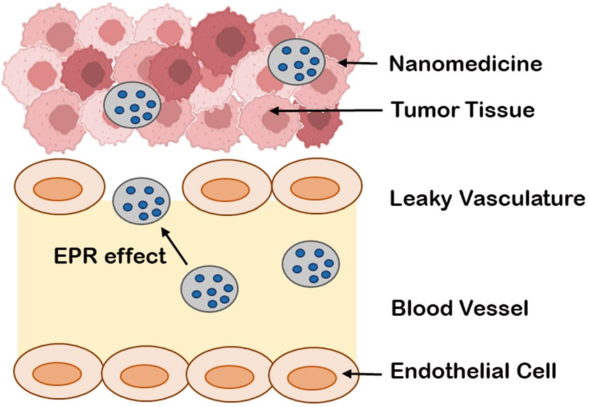


Fig. 2 Passive Targeting: EPR effect facilitating passive targeting

ligand-receptor is internalized by the plasma membrane to form an endosome, by the mechanism of receptor-mediated endocytosis. The newly developed endosome is transferred to particular organelles, where enzymes or an acidic pH may release drugs [6]. Nano drugs do not have a particular target in the current scenario of clinical development. To examine the enormous potential of actively targeted drug delivery, we must discover true targets in diseases. The site or the mode of action of therapeutic drugs, their properties should be appropriate to target and the delivery vehicles are best for the development of new drugs [111] (Fig. 3).

Stimuli-Activatable Strategies

Compared to traditional nano-range materials, stimuli-responsive nanoparticles show enormous potential for cancer diagnostics and therapy. The use of light-responsive nanomedicines for PDT, in which photosensitizers encapsulated in nanomaterials accumulate in tumor tissues passively respond to specific wavelengths of light, is one such example of a stimuli-responsive approach in cancer tumor targeting. This method ultimately induces oxidative damage to tumor cells, leading to necrosis or apoptosis [105]. Therefore, exposure to certain stimuli is a fundamental component of a responsive drug delivery system, and changes in nanoparticle structure or composition of nanoparticles results in controlled release of drugs. These stimuli can be internal or external.

Internal Stimuli-Responsive Systems

Certain features are specific to the tumor microenvironment (TME) as compared to normal cells or tissue that can be used in the development of internal-stimuli-sensitive drug delivery systems. Numerous instances exist of internal stimuli systems that make use of temperature, redox systems, pH, and enzyme activity [106,

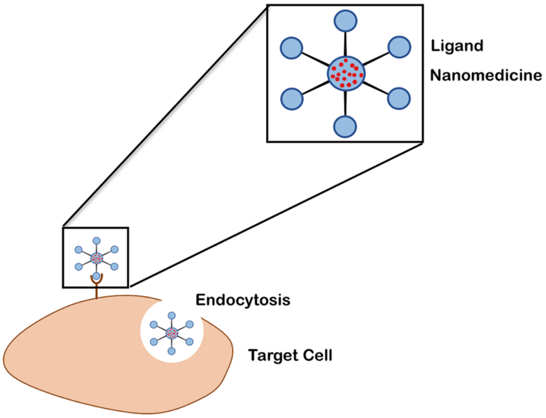


Fig. 3 Active targeting: Nanomedicine attached to ligand binds to the target cell's receptor resulting in endocytosis of the target cell

109]. pH-sensitive NPs flowing across the circulatory system are usually stable at physiological pH. Upon deposition into a tumor, an acidic TME is generated which causes the loading of the bioactive thus resulting in accomplishing pH-responsive drug delivery systems, by covalently attaching anticancer drugs to biocompatible polymers [113, 114]. Utilizing the difference in temperature between a tumor and a healthy cell, temperature-responsive nanomedicine can also be designed to release anticancer medications that are encapsulated by altering their structural composition [109]. For the regulated release of anticancer medications, systems with chemical and physical characteristics that respond to the biocatalytic reactions of tumor-specific enzymes may be employed [109, 115].

External Stimuli-Responsive Systems

Due to their non-invasive nature and the ability to manage medication delivery spatiotemporally, external stimuli-responsive systems, such as light-responsive systems can be divided into three types: photothermal therapy (PTT), photodynamic therapy (PDT), and light-triggered cargo release. These are advantageous due to their non-invasiveness and non-ionization in the drug administration process. Ultrasound-responsive drug delivery systems are becoming progressively popular because of the benefits similar to those of light-responsive systems. A durable and effective magnetically responsive formulation is used in magnetic field-assistant medication delivery to increase drug loading and inhibit tumor formation. To target neoplasms, tumor-targeted immunotherapy modifies the host's immunity by employing strategies such as immune checkpoint blockers, adoptive cell therapies, and therapeutic vaccines that have demonstrated impressive clinical responses [109].

4 Applications

In the previous few decades, nanotechnology has been increasingly prevalent in the majority of nanomedicine-related subfields for therapeutic and diagnostic clinical applications. The potential use of gold nanoparticles (AuNPs) in medicine for the diagnosis and treatment of a wide range of illnesses is demonstrated by their interactions with numerous physiologically active substances [116]. Various studies have focused on determining if AuNPs may be used to diagnose and treat a variety of illnesses, such as cancer, metabolic disorders, and neurodegenerative diseases [116]. A variety of materials have been made and, based on their physicochemical properties, explored for their potential benefits as a promising alternative for controlled pharmacological loading to enhance therapeutic efficacy and stability [117].

4.1 In Vitro *Applications*

Nanoparticles as nanomedicine are comprehensively studied *in vitro* for various purposes involving utilizing nanoparticles and nanostructures, typically within controlled laboratory settings and clinical environments for drug development and screening, biosensing, bioimaging, diagnostics, and therapeutics.

Drug Development and Screening

Since 2010, the number of marketed nanomedicines has increased significantly, and it is expected that pharmaceutical nanomedicine products will have a substantial impact on the worldwide pharmaceutical sector [118]. Extensively utilized in the field of oncology, nanomedicine has gathered notable interest in solving the present clinical and pharmacological obstacles of traditional treatment approaches. It is with their tested and clinically approved formulations of cancer nanomedicines based on either polymeric micelles, encapsulating hydrophobic therapies, albumin, nanocrystals or inorganic nanoparticles, which in clinical practice have shown to improve the rates of survival [119, 120]. While cancer nanomedicines currently make up the largest portion of all drug classes available on the market, many formulations are also being marketed for other indications, such as inflammatory diseases, neurological disorders, autoimmune conditions, metabolic disorders, ophthalmic conditions, and hematological disorders [118]. The treatment of bacterial, fungal, and viral infections has also made use of a variety of nanoparticle-based techniques, including liposomal amphotericin B, cabotegravir/ rilpivirine extended-release nano-suspension, and mRNA-lipid nanoparticles [65, 117]. Lipid nanoparticles (LNPs) as non-viral vectors have been employed significantly due to their potential in *in vivo* mRNA delivery. mRNA-LNPs have emerged recently for COVID immunizations such as mRNA-1273 (Moderna) and BNT162b2 (Pfizer-BioNTech), with the intent to prevent SARS-CoV-2 which have been granted emergency use authorization (EUA) by US FDA [118]. Candidate drug screening along with the development is also essential and may be greatly aided by using QDs as an inventive fluorescence probe for drug screening [121]. The detailed list of clinically approved nanomedicines by the Food and Drug Administration (FDA) and European Medicine Agency (EMA) is given in Table 1.

Smart Sensing

Nanosensors capable of measuring the body's levels of carbon dioxide and oxygen as well as the presence of dangerous substances, malignant digestive organs, and food sensitivity are used [122]. In recent years, Magnetic Nanoparticles (MagNPs) have been effectively used as labels in magnetic nanomaterial-based sensing systems (micro-Hall, giant magnetoresistive sensors) are employed in medical imaging

Table 1 List of globally marketed nanomedicines approved by the FDA and the EMA[a]

Type	Trade Name	Company	Date of Approval	Active Ingredients	Indication
Nanocrystals	Emend®	Merk & Co. Inc.	FDA (2003)	Aprepitant	Antiemetic drug
	Ivemend®	Merk & Co. Inc.	FDA, EMA (2008)	Fosaprepitant dimeglumine (prodrug of aprepitant)	Antiemetic drug
	Ostim®	Osartis GmbH & Co.	FDA (2004)	Calcium hydroxyapatite	Bone-grafting material
	Rapamune®	Wyeth Pharmaceuticals Inc. (a subsidiary of Pfizer Inc.)	EMA (2001), FDA (2010)	Sirolimus (rapamycin)	Prevents rejection of kidney transplants (immunosuppressant)
	Rapamune®	Wyeth pharmaceuticals Inc. (a subsidiary of Pfizer Inc.)	FDA (2015)	Sirolimus (rapamycin)	a rare progressive lung disease (lymphangioleiomyomatosis)
	Vitoss®	Orthovita Inc.	FDA (2003)	β-tricalcium phosphate	Bone-grafting material
	Ritalin LX®	Novartis	FDA (2002)	Methylphenidate	Attention deficit hyperactivity disorder (ADHD) in children
	Avinza®	Pfizer Pharmaceuticals	FDA (2002)	Morphine sulfate	Psychostimulant
	Focalin XR®	Novartis	FDA (2008)	Dexamethylphenidate HCl	ADHD in children
	Invega®	Janssen Pharmaceuticals	FDA (2009)	Paliperidone	Schizophrenia
	Invega Sustenna®	Janssen Pharmaceuticals	FDA (2009)	Paliperidone Palmitate	Schizophrenia
	Megace ES®	Par Pharmaceuticals	FDA (2005)	Megestrol acetate	Antianorexic

	NanOss®	RTI Surgical	FDA (2005)	Hydroxyapatite	Bone substitute
	EquivaBone®	Zimmer Biomet	FDA (2009)	Hydroxyapatite	Bone substitute
	OsSatura®	Isotis Othobiologics Inc.	FDA (2003)	Hydroxyapatite	Bone substitute
	Epaxal®	Crucell Berna Biotech	EMA (1993)	Inactivated hepatitis A virus vaccine	Prevents hepatitis A infection
	Zanaflex®	Acorda	FDA (2002)	Tizanidine HCl	Muscle relaxant
	Ryanodex®	Eagle pharm	FDA (2014)	Dantrolene sodium	Malignant hyperthermia
	TriCor®	Abbott Laboratories	FDA (2004)	Fenofibrate	Antihyperlipidemia
Lipid-based nanoparticles	Doxil®	Johnson & Johnson	FDA (1995), EMA (1996)	Doxorubicin (adriamycin)	Metastatic ovarian cancer, HIV-associated Kaposi's sarcoma (KS)
	Lipodox®	Sun Pharma Global FZE	FDA (2013)	Doxorubicin hydrochloride	Metastatic ovarian cancer, HIV-associated KS
	DaunoXome®	Galen Ltd.	FDA, EMA (1996)	Daunorubicin	Cancers and HIV-associated KS
	Onivyde®	Merrimack Pharmaceuticals	FDA (2015)	Irinotecan	Metastatic pancreatic cancer

(continued)

Table 1 (continued)

Type	Trade Name	Company	Date of Approval	Active Ingredients	Indication
	DepoCyt®	Pacira Pharmaceuticals	EMA (2002), FDA (2007)	Cytarabine	Lymphomatous meningitis
	Myocet®	Teva Pharmaceutical Industries Ltd.	EMA (2000)	Doxorubicin hydrochloride	Breast cancer
	Caelyx®	Janssen Pharmaceuticals	EMA (1996)	Doxorubicin	Breast cancer, ovarian cancer, HIV-associated KS
	Mepact®	Takeda France SAS	EMA (2009)	Mifamurtide	Osteogenic sarcoma
	Marqibo®	Talon Therapeutics	FDA (2012)	Vincristine	Philadelphia chromosome-negative chronic myelogenous leukemia in adult patients
	Onpattro®	Alnylam	FDA & EMA (2018)	Patisiran	Hereditary transthyretin (TTR) mediated amyloidosis
	Lipusu®		FDA (2016)	Paclitaxel	Breast cancer, non-small-cell lung cancer (NSCLC)
	AmBisome®	NeXstar Pharmaceuticals	EMA (1990), FDA (1997)	Amphotericin B	Antifungal drug
	Vyxeos®	Jazz Pharmaceutics	FDA (2017), EMA (2018)	Daunorubicin and cytarabine	Acute myeloid leukemia
	Abelcet®	Defiante Farmaceutica	FDA (1995)	Amphotericin B	Antifungal drug

	DepoDur®	SkyePharma	FDA (2004), EMA (2006)	Liposomal morphine sulphate	Postoperative analgesia
	Curosurf®	Chiesi	FDA (1999)	Poractant alfa	Respiratory distress syndrome (RDS)
	Zevalin®	Bayer Pharma	FDA (2002) Disc.[a] EMA (2004)	90Y-ibritumomab tiuxetan	Lymphoma
	Inflexal®	Crucell Berna Biotech	EMA (1997)	Inactivated influenza virus vaccine	Prevents influenza infection
	Pfizer-BioNTech vaccine	Pfizer pharmaceuticals	FDA (2020)	mRNA vaccine	Prevents COVID-19 infection
	Moderna COVID-19 Vaccine	ModernaTX Inc.	FDA (2020)	mRNA vaccine	Prevents COVID-19 infection
	Visudyne®	QLT Phototherapeutics	FDA & EMA (2000)	Photosensitizer (PS), benzoporphyrin	Choroidal neovascularization caused by wet age-related macular degeneration

(continued)

Table 1 (continued)

Type	Trade Name	Company	Date of Approval	Active Ingredients	Indication
Polymer-based Nanoparticles	Cimzia®	UCB	FDA (2008), EMA (2009)	IgG Fab' fragment that specifically recognizes and binds to TNF-α	Rheumatoid arthritis, Crohn's disease, psoriatic arthritis, and ankylosing spondylitis
	Apealea®	Oasmia Pharmaceutical AB	EMA (2018)	Paclitaxel	Ovarian cancer, peritoneal cancer, fallopian tube cancer
	Adagen®	Enzon Pharmaceuticals Inc.	FDA (1990)	Adenosine deaminase (ADA)	Adenosine deaminase (ADA)-severe combined immunodeficiency disorder
	Neulasta®	Amgen, Inc.	FDA (2002)	Filgrastim	Febrile neutropenia, consequent infections arising due to lack of neutrophils
	Oncaspar®	Enzon Pharmaceuticals Inc.	FDA (1994), EMA (2016)	L-asparaginase	Acute lymphoblastic leukemia, chronic myelogenous leukemia
	Genexol-PM®	Lupin Ltd.	FDA (2007)	Paclitaxel	Breast cancer
	Pegasys®	Genentech USA, Inc	FDA, EMA (2002)	Recombinant human alfa-2a interferon	Hepatitis C, hepatitis B
	Diprivan®	Fresenius Kabi	FDA (1989), EMA (2001)	Propofol	(sedative-hypnotic agent) used in surgery to induce relaxation before and during general anesthesia
	Somavert®	Pfizer Pharmaceuticals	EMA (2002), FDA (2003)	Analog of human growth hormone (acts as an antagonist of GH receptors)	Acromegaly

	Macugen®	Pfizer Pharmaceuticals	FDA (2004)	Pegaptanib sodium	Choroidal neovascularization caused by wet age-related macular degeneration
	Mircera®	Vifor	EMA (2007), FDA (2018)	Epoetin β (EPO) (EPO is a genetically recombinant form of erythropoietin)	Anemia
	PegIntron®	Merk & Co. Inc.	EMA (2000), FDA (2001)	Alpha interferon (INF) molecule	Hepatitis C
	Krystexxa®	Savient Pharmaceuticals	FDA (2010)	Pegloticase is a recombinant porcinelike uricase	Refractory chronic gout
	Plegridy®	Biogene	FDA (2014)	Recombinant IFN-β	Relapsing remitting multiple sclerosis (RRMS) in adult patients
	Adynovate®	Baxalta US Inc.	FDA (2015)	Coagulation factor VIII	Hemophilia A
	Copaxone®/FOGA	Teva Pharmaceutical Industries Ltd.	FDA (1996), EMA (2016)	Glatiramer acetate	Multiple sclerosis (MS)
	Eligard®	Tolmar Pharmaceuticals Inc.	FDA (2002)	Leuprolide acetate	Prostate cancer
	Renagel®	Sanofi	FDA (2000)	Sevelamer carbonate	Hyperphosphatemia caused by chronic kidney disease (CKD)
	Renagel®/ Renvela®	Genzyme	EMA (2007)	Sevelamer HCL	Hyperphosphatemia caused by CKD

(continued)

Table 1 (continued)

Type	Trade Name	Company	Date of Approval	Active Ingredients	Indication
	Restasis®	Allergan	FDA (2003)	Cyclosporine	Chronic dry eye
	Rebinyn®	NovoNordisk	FDA (2017)	Recombinant DNA-derived coagulation FIX	Hemophilia B
	Estrasorb™	Novavax, Inc.	FDA (2003)	Estradiol (17β-estradiol) hemihydrate	Moderate vasomotor symptoms due to menopause
	Zilretta®	Flexion Therapeutics	FDA (2017)	Triamcinolone acetonide	Knee osteoarthritis
Dendrimer-based Nanoparticles	VivaGel® BV	Starpharma	FDA (2015)	Astodrimer sodium	Anti-infective for prevention of recurrent bacterial vaginosis (BV)
Protein-based Nanoparticles	Abraxane®	Celgene Pharmaceutical Co. Ltd.	FDA (2005, 2012, 2013), EMA (2008)	Paclitaxel	Approved by the FDA for treatment of metastatic breast cancer (2005), lung cancer (2012), and metastatic pancreatic adenocarcinoma (2013)
	Ontak®	Eisai	FDA (1999)	Diphtheria toxin	Leukemia, T-cell lymphoma

Inorganic nanoparticles	Feraheme™	AMAG Pharmaceuticals	FDA (2009)	Ferumoxytol	Anemia
	Venofer®	Luitpold Pharm	FDA (2000)	Iron sucrose	Iron deficiency in CKD
	Dexferrum®	American Regent	FDA (1996)	Iron dextran	Iron deficiency in CKD
	Ferinject®	Vifor	FDA, EMA (2013)	Iron carboxymaltose colloid	Iron deficient anemia
	Ferrlecit®	Sanofi-Aventis	FDA (1999), EMA (2013)	Sodium ferric gluconate	Iron deficiency in CKD
	Hensify®	Nanobiotix	EMA (2019)	Hafnium oxide nanoparticles	Locally advanced squamous cell carcinoma
	Infed®	Actavis Pharma	FDA (1992)	Iron dextran	Iron deficiency in CKD
	Feridex®/Endorem®	AMAG Pharma	FDA (1996) Disc.[a] 2008	SPION-dex	Imaging agent
	GastroMARK™/Umirem®	Mallinckrodt Inc.	FDA (2009) Disc.[a] 2012	SPION-silicone	Imaging agent

Adapted from Halwani [118]. Reproduced vide [CC-BY-NC-ND 4.0]

[a] Disc: discontinued

as well as magnetic particle spectroscopy bioassays, in the field of magnetic bioassay where the MagNPs are enabled with ligands (such as antibodies, proteins, and ligands) which can identify and attach to target analytes specifically [123]. QDs are widely used as luminescent transducers in the development of optical biosensors and cellular biomarkers to develop intracellular sensors, such as pH sensors, which could measure intracellular pH, which is frequently linked to diseases like cancer and Alzheimer's disease [124].

Bioimaging

Nanotechnology-based bioimaging has advanced significantly in recent years. QDs have demonstrated significant promise in the realm of biological medicine. *In vitro,* labeling by QDs has played a crucial role in molecular and cell-tissue imaging due to their brilliant fluorescence, broad UV excitation, narrow emission, and great photostability. Luminescent QDs, or semiconductor nanocrystals, have been shown as a promising substitute for organic dyes in molecular targeting over conventional fluorescent dyes and proteins. There are several distinct kinds of QDs, each with unique characteristics and chemical compositions. For instance, in gastric cancer, CA 72-4 can be quickly and quantitatively detected using lateral flow strips labeled with CdSe/ZnS QDs [121]. The early detection of cancer has been now made possible by using immunological superparamagnetic iron oxide nanoparticles (SPIONs) as the target during MRI imaging of lung cancer cell [11]. The development of room-temperature biocompatible Magnetoelectric nanoparticles (MENPs) has permitted the formation of a wireless two-way interface between physiological systems and synthetic devices at the molecular level. This has led to the emergence of several novel potential medical applications, such as real-time mapping and monitoring of brain activity, personalized medication administration across the blood-brain barrier (BBB), tissue repair, highly specialized cancer treatments, quick molecular diagnostics, etc. [125]. AuNPs have been studied in several other *in vitro* experiments and have been used in various bioimaging methods such as photothermal phase microscopy, photoacoustic imaging, computed tomography of X-ray fluorescence, and three-photon luminescence [116].

Diagnostics

In addition to conventional biomarker-based techniques, array-based "chemical nose" sensors have been developed for the specific identification of analytes, including the detection of sera, the differentiation of bacteria based on their cell surfaces, and the genotyping of cancer cells [124]. Embedded nanofibers-based intelligent bandages have now been developed to include coagulation, antibiotics, and even sensors to identify infection symptoms [122]. Barcodes for biological applications that have been surface-coated with biorecognition molecules (such as antibodies or oligonucleotides) may be used to identify the genetic targets of hepatitis B and HIV patients [124]. The precision, adaptability, and sensitivity of Ovarian Cancer (OC) biomarker detection have been greatly enhanced using nanomaterial-based *in vitro* diagnosis. Analysis of extremely low concentrations of circulating tumor cells and exosomes in OC blood is made feasible by nano-pattern enclosed microfluidic chips, multi operational electrochemical detection, and magnetic separation devices [126].

4.2 In Vivo *Applications*

In vivo applications of nanomedicine involve utilizing nanoparticles and nanostructures for medical purposes within living organisms for advancing the diagnosis, treatment, and monitoring of various diseases due to their unique properties such as effective targeted drug delivery, cancer, and gene therapy, theragnostics, regenerative medicine, imaging, etc.

Particle Size Drug Delivery and Treatment

Several forms of nanoparticle-based drug delivery systems are frequently used, including micelles, dendrimers, lipid-based nanoparticles, protein nanoparticles, polymer-drug conjugates, nanocapsules, nanoemulsions, and inorganic nanoparticles collectively known as pharmaceutical nanocarriers (Fig. 4).

Organic and inorganic nanoparticles broadly classified into three classes such as polymer-based, lipid-based, and dendrimers are very desirable for use in drug administration and are also known to be functionalized to enhance the medicinal medicines' pharmacokinetic characteristics [118]. Polymeric nanoparticles (PNPs), also referred to as polymer nanoparticles, nanospheres, nanocapsules, or polymer micelles, were the first polymers to be reported for use in drug delivery systems for hydrophobic drugs because of their smaller volume and higher thermodynamic stability, making them excellent drug carrier that could prevent kidney rejection while maintaining good endothelial cell permeability [11]. Polymeric nanomedicines, comprising biocompatible and biodegradable polymers, such as chitosan, formulated with hydrophilic molecules such as PEG, succinic anhydride, or β cyclodextrin enhance the solubility and stability of chitosan-based polymeric nanoparticles in mildly acidic or alkaline environments resulting in the development of effective drug-delivery systems for treating bacterial infections [127]. With their potential to conjugate or encapsulate therapeutic substances, these permeability-enhancing chitosan nanoparticles are especially well-suited for drug delivery *via* pre-oral, nasal, pulmonary, buccal, vaginal, and rectal routes. These polymer-based nanocarriers offer an option for intranasal medication administration or brain delivery to enhance patients' adherence to therapy because of their mucoadhesive properties [127, 128]. Inorganic and organic NPs also tend to provide a novel approach to unfulfilled clinical requirements, particularly in the ophthalmic domains. Liposome-based NPs

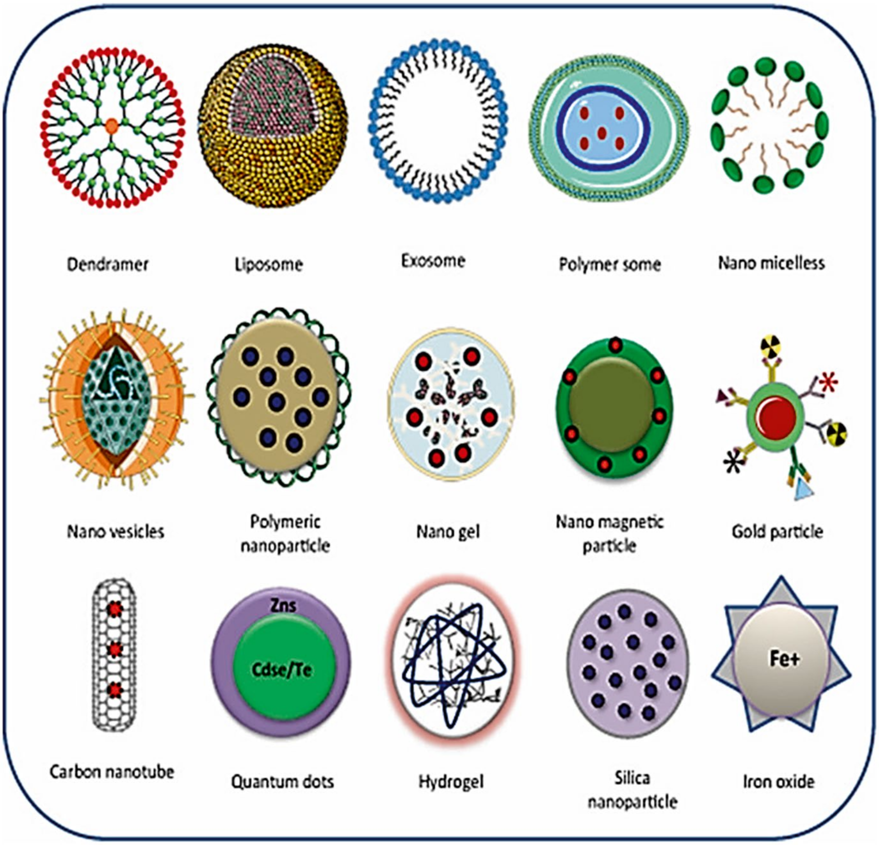
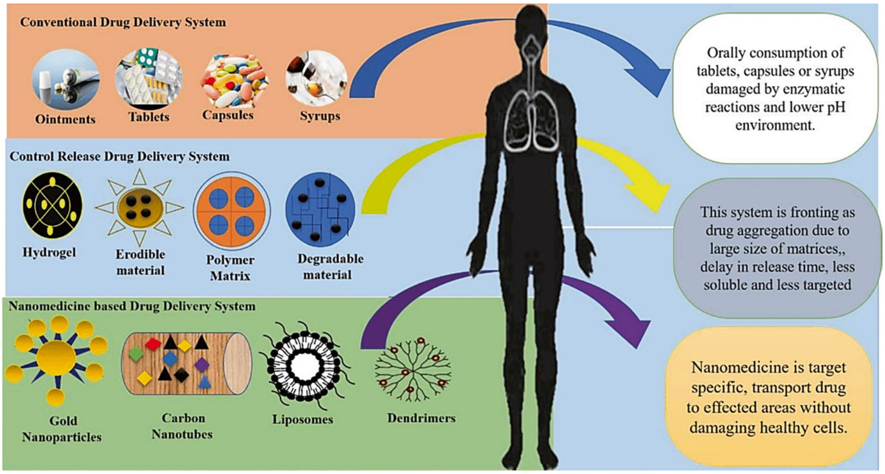

Dendramer
Liposome
Exosome
Polymer some
Nano micelless
Nano vesicles
Polymeric nanoparticle
Nano gel
Nano magnetic particle
Gold particle
Carbon nanotube
Zns
Cdse/Te
Quantum dots
Hydrogel
Silica nanoparticle
Fe+
Iron oxide

Fig. 4 Different types of nano-molecules employed in drug delivery and treatment [133]. (Reproduced with permission 5756940390125)

offer potential in ocular drug delivery as their surface may be altered for targeted distribution, leading to significant improvement in drug absorption in the posterior region of the eyes. Furthermore, the positive charge of the liposome may also increase medication penetration into the cornea, prolonging drug release and reducing the need for recurrent drug administration (Fig. 5) [128]. Liposomes have also been extensively researched as targeted drug therapy for blood cancers, micrometastases, and the vascular system as they can internalize drugs into the intracellular space through endocytosis when fused with tumor cells, and release drugs through passive or active ligand-mediated activity [11, 129]. Niosomes, a separate kind of organic NPs, were initially extensively employed in the cosmetics sector, but their potential for drug delivery was later investigated for various administration methods. Other forms of organic NPs, such as dendrimers have also attracted a lot of attention in the pharmaceutical industry due to their unique architecture they are an excellent choice for nanocarriers for ophthalmic drug delivery as well as other



Orally consumption of tablets, capsules or syrups damaged by enzymatic reactions and lower pH environment.

This system is fronting as drug aggregation due to large size of matrices,, delay in release time, less soluble and less targeted

Nanomedicine is target specific, transport drug to effected areas without damaging healthy cells.

Fig. 5 Illustration of administration of traditional medications in contrast with modern procedures using nanomedicines to transport medication to specific parts of the body [131] (Reproduced vide CC-BY-NC-ND 4.0)

medical domains because of their capability to improve permeability, water solubility, biocompatibility, and bioavailability [128]. In contrast to matrix NPs like polymeric or lipid matrices, another class of NPs known as nanocrystals has also been used in drug delivery because of their high drug loading (almost 100%), which permits high drug concentration at the site of action and decreases the potential toxicity of the excipients [130].

With its unique ability to form cross-links with biomolecules, such as small-molecule ligands, proteins, and antibodies, Quantum dot technology has attracted a lot of attention in the pharmaceutical and pharmacological fields. This property makes QDs highly desirable for targeted medication delivery and biological system diagnostics. Near-infrared QDs linked with magnetic nanospheres (MNSs) made up of spherical polystyrene matrix encapsulating Fe_3O_4 NPs on the surface can be used to specifically target biological systems [121]. Organic nanotubes have been observed to enhance biofilm penetration into living cells without causing any invasion, making them highly effective transporters. As they are compatible with anticancer drugs like paclitaxel, they can be administered both *in vivo* and *in vitro*, resulting in effective cancer treatment [11]. Inorganic NPs like mesoporous silica nanoparticles (MSNs) have also been investigated as drug delivery systems because of their hydrophilic nature, low toxicity profile, and biocompatibility, rendering them appropriate for biological applications, especially in the field of oncology [132, 133]. Given their widespread application in nanomedicine, inorganic nanoparticles like gold and silver (Gold nanoparticles (AuNPs) and Silver Nanoparticles (AgNPs)) are employed as nanospheres, nanorods, nanostars, nanoshells, and nanocages, among other forms making them excellent candidates as drug delivery vehicles [134]. AuNPs have been exploited for their use in the treatment of rheumatoid arthritis as well as drug delivery vectors to treat malignancies [116]. Furthermore, AgNPs have demonstrated efficaciousness as therapeutic delivery systems in ophthalmology, in addition to their applicability in the field of oncology. Whether used solely or in combination with plant extracts, AgNPs have demonstrated enhanced antioxidant, anticataractogenic, and anti-angiogenic effects in the study of various ocular diseases *in vivo* [128, 135]. AgNP synthesis from plants has significantly advanced recently and has enormous potential for treating diseases like cancer, HIV/AIDS, fungal and bacterial infections, and wound-healing applications [117]. In addition to these cellular processes, AgNPs have also demonstrated anti-proliferative and anti-angiogenic qualities [65]. Additionally, it has been reported that certain fluorescent molecules, including proteins, growth hormones, siRNA molecules, DNA molecules, antibodies, and other molecules, when conjugated to the Nanodiamonds (NDs), a class of carbon nanoparticles, are useful in the fields of targeted drug delivery, cancer treatment, protein separation, and purification, etc. [113]. Magnetic Nanoparticles (MNPs) have also been widely exploited as small carriers for drug/gene delivery in magnetic therapeutic applications [123]. Gelatin-based hydrogels are widely utilized in drug delivery, tissue regeneration, cell culture, wound casting, and other applications and are commonly mixed with supportive elements such as chitosan, alginate, carbon nanotubes, and polyvinyl alcohol. They have been extensively used to target the tissues injured by malignancies, TB, and HIV infections in conjunction with the therapy of vasospasm and restenosis [136]. Ribonucleic acid (RNA) nanotechnology, a promising platform for drug delivery applications, akin to protein carriers has been reported to target, image, deliver, and silence genes, and slow the progression of stomach cancer making it one of the emerging novel technologies in oncology [124]. Modern healthcare advancements have culminated in improved CNS disease treatment and management regimes. However, some conditions, including Parkinson's, epilepsy, stroke, neuroAIDS, and Alzheimer's disease, never entirely recovered from. These conditions are rising in frequency across the globe along with cancer incidences. For CNS drug delivery applications, a variety of NPs have been investigated, including NCs gold, silica, hydrogels, liposomes, magnetic NPs, magnetoelectric NPs (MENPs), etc. [137]. As nanocarriers, exosomes have also shown significant promise for a variety of therapeutic applications, such as cancer and inflammatory diseases, as well as for diagnostic purposes.

Tissue Engineering

Specifically utilized as scaffolds in cases of trauma, injury, or certain diseases, such as cancer, osteoporosis, and degenerative disorders, scaffolding using hydrogels have been shown to prevent pathogen transfer and immune reactivity (Fig. 6 and Table 1) [136]. The advent of nanobots, which can either repair damaged cells or completely alter intracellular components, was a noteworthy advancement in the field of nanomedicine. They were employed in restoring the genetic defects or substituting a new DNA molecule, which might be used to eradicate the illness [122].

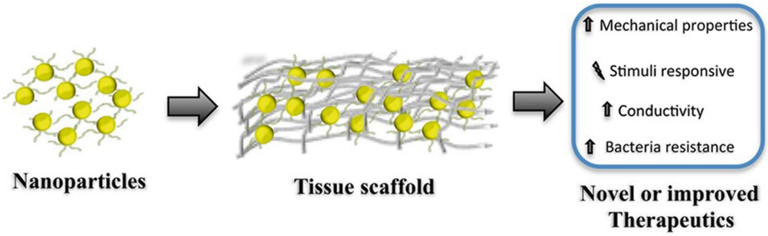


Fig. 6 Schematic illustration for the development of hybrid regenerative tissue scaffolds with NPs [153]. (Reproduced vide CC-BY-NC-ND 4.0)

The excellent mechanical properties of nanodiamond (NDs) polymer composites, which are used to reinforce implantable polymers to create multifunctional scaffolds for tissue engineering, make them particularly promising for the restoration of damaged tissue in tissue engineering and regenerative medicine [124].

Despite several medical advancements, heart failure, including myocardial infarction (MI), and cardiovascular disease (CVD) continue to be the primary causes of morbidity and mortality in developed nations. Heart tissue engineering has recently become known as a potentially effective method for producing cardiac grafts, which can be whole heart replacements or tissues that can be successfully transplanted in an organism, regenerating tissues for a fully functional heart without adverse effects like immune rejection. For cardiac tissue engineering, collagen, and fibrin scaffolds, particularly nanofibrous scaffolds, have been studied. The behavior and growth of cardiomyocytes in collagen hydrogels fabricated using carbon nanotubes have also been assessed [138]. In a wide number of disciplines, including material science and biomedical engineering, nanocellulose has recently attracted more attention. A promising biomaterial, nanocellulose is naturally suited for tissue engineering supports and can be used to fabricate a variety of morphologies, including membrane-like structures, hydrogels, and electrospun fibers with tunable surface chemistries and porosities [139]. The new porous collagen scaffold has the potential to improve dermal regeneration in traumatic skin wounds that would impede the normal healing process [140].

Theragnostics

Applications of many types of nanomaterials in theragnostic, including the use of polymeric, liposomal, metal, and bio-nanoparticle nanoparticles in theragnostic have demonstrated potential for lung cancer treatment and diagnostics [141]. Novel strategies for treating cancer have been put forth, such as magnetic nanoparticle-induced Magnetic Hyperthermia (MHT) to kill cancer cells efficiently [118].

Molecular Imaging

In light of bioimaging techniques, hybrid inorganic nano heterogeneous structures as contracting agents have been developed which are based on the combination of different domains, exhibiting different functionalities, and being available for use in various diagnostic or therapeutic techniques, such as Positron Emission Tomography (PET), CT, MRI, and X-ray-CT, that provide more details about the tissues, organs, or changes in metabolism [142]. Magnetic Nanoparticles have already been used in magnetic resonance imaging (MRI Magnetic Particle Imaging) as contrast enhancers mainly for chronic kidney disease patients [123]. Apart from the *in vitro* utility of QDs, the most recent generation of QDs, also called engineered QDs, have superior optical characteristics and a distinctive near-infrared (NIR) fluorescence that makes them compatible with in vivo applications like whole body, tumor, vasculature, and lymph node imaging includes Non-Cd QDs and NIR-II window QDs like InAs/InP/ZnSe Core/Shell/Shell QDs to name a few [121]. Through research and experimentation, scientists have also demonstrated explicitly that QDs can be used for imaging of mouse embryonic stem cells and adipose tissue-derived stem cells for cell tracing after transplantation having implications for regenerative medicine [121]. Nanodiamonds (NDs), a novel class of carbon nanomaterials have recently shown potential as contrast agents when compared to other nanomaterials, making them a good option for imaging cancer cells [143].

5 Challenges & Limitations

5.1 Challenges

Indeed, nanomedicine has garnered substantial research interest over time due to its potential therapeutic application. These applications range from formulation to targeting strategies. The advantages of nanomedicine, such as its small size, lower dosage requirements, and enhanced bioavailability have fueled its interest. But, commercial and practical feasibility, along with affordability, remain a concern [144]. Regulatory bodies such as the FDA and EMA have established guidelines for nanomedicine, however, the formulation and characterization of multifunctional nanomaterials require careful consideration of parameters such as pH, light sensitivity, thermostability, dilutant requirements, and lyophilization [145, 146]. For example, active targeting of bacterial infection presents several challenges, primarily due to the immunostimulatory nature of bacteria. Components (flagella, lipopolysaccharide, etc) of bacteria can induce an immunogenic response, accelerating the immune response. Consequently, the management of bacterial infection requires careful consideration of oral formulation for therapeutic peptides and nucleic acid. These formulations need to be studied in acidic condition (gastric) and for intestinal membrane penetration. Additionally, parameters such as fail-safes (like kill switches and genetic firewalls) and appropriate manufacturing techniques are necessary. In the context of multi-drug resistance (MDR) infection, the challenges over the administration of NP drugs are toxicity and identification of routes for administration. It is important as NP may accumulate in the spleen, lung, and bone marrow if administered intravenously and their small size allows distribution in multiple organs potentially leading to nanotoxicity. Furthermore, NPs have been observed to induce oxidation stress which can lead to hepatotoxicity and lung toxicity and may modify metabolic pathways.

In the context of passive targeting for cancer treatment, liposomes are nanostructures self-assembled from a combination of phospholipids and cholesterol compounds akin to the cell plasma membrane enabling them for effectively distribution through hydrophobic bilipid membrane. However, liposome-based liver cancer models have encountered challenges due to the degradation of liposomes via hydrolysis and oxidation, which could be attributed to bodily variations or drug instability. Therefore, it is imperative to analyze and optimize certain factors for improved payload delivery. On the other hand, Colon targeted delivery systems (CDDS) have exhibited a weak chemotherapy response and low drug payload wherein the payload is disrupted before reaching the target site. This necessitates the development of a multi drug resistance phenotype with enhanced drug translation.

5.2 Limitations

The accelerated advancements in nanomedicine have enabled control over multiple parameters for manufacturing and development. However, a conspicuous absence of standardization persists [147, 148]. Furthermore, humans have exhibited resistance to certain nanomaterials that have received approval from the FDA for use in nanomedicine [149]. Concurrently, several studies have scrutinized the physicochemical properties and reproducibility of nanomedicine from the perspective of clinical application [150, 151]. Despite the benefits of combination therapy in combating bacterial infections, the integration of nanomedicine institutes certain concerns [152]. Following are some of the limitations of the application of nanomedicine:

1. Renal pathogenesis, including chronic kidney diseases (CKD) and diabetic kidney diseases (DKD), has yet to receive clinical approval due to a multitude of factors. These renal diseases are associated with various elements such as diabetes, metabolic factors, and daily consumption habits, among others. The utilization of extracellular vesicles (EVs) as targeted therapeutic agents is significantly variable and is unstandardized. Consequently, the targeted study is currently underway.
2. In content of dendrimer-based nanoparticles, Polyethylene glycol (PEG) models have received FDA approval. However, the emergence of Anti-PEG antibodies has been observed in humans. For instance, pegnivacogin, an RNA aptamer

conjugated with methoxy PEG (mPEG), may yield unexcepted results when administrated to the anti-PEG antibody population, which constitutes nearly 70% of untreated individuals. This occurrence leads to a loss of pharmaceutical activity.

3. Regarding barriers to development, the routes of administration, both non-invasive and invasive (such as intrathecal and intra-hippocampal injection), pose ethical challenges for clinical development. Similarly, diseases and disorders such as diabetes and diabetic foot ulcer (DFU) present challenges due to physio-chemical and reproducibility factors associated with nanomaterials. Furthermore, the long-term effects of these nanomaterials on the human body are yet to be understood, as therapeutic response mapping is currently limited to mice and rat models.
4. In the context of combinational strategies, certain aspects of bacterial infection management remain to be studied. These include the synergistic action mechanism of nanomaterials in combination therapy with drugs, the accumulation of nanomaterials in lesions, and the metabolic pathways for the clearance of bacterial diseases. Additionally, the potential toxicity of these treatments on vital organs is a significant concern. Furthermore, the implementation of phototherapy associated nanomedicine is contingent upon the specific identification of the infection site. These represent some of the limitations associated with the treatment of bacterial infections using these strategies.

6 Prospects

Nanomaterials can be beneficial for many facets of human existence. Additional studies in nanotechnology and the reasonable integration of nanomaterials into the current therapies domains such as nutraceuticals, stem cell research, and regenerative medicine are likely to be impacted by advances in nanotechnology shortly [153]. These applications range from efficient drug delivery to targeting multiple diseases. The administration of a theragnostic agent (nanomedicine) could potentially allow monitoring of patient response. Consequently, this nanomedicine should also enable the real-time visualization of the effect of the drug. Certain prospects which we believe need attention from the research community are articulated here:

1. In the realm of microbial infection control, it is crucial to broaden the scope of microbiome magnetic nanoparticles (MNPs) applications, while also refining their production and use in biomaterials. The investigation of innovative MNPs or the integration of MNPs with various biomaterials can be utilized at different stages of diabetic wound healing to maximize functionality and minimize potential complications [154].
2. About neuropathy, diabetic peripheral neuropathy (DPN), there is a need for more stable, effective, and high bioavailability nanomedicine that provides analgesia effect throughout the human body [150]. The field of encapsulation, where

controlled drug release and semi-permeability are necessary, requires further scientific research for application in diabetes and cardiovascular diseases [155]. Furthermore, the bioavailability of nanomedicine, targeted therapeutic and anti-release nanozymes are considered the scope of future development [151].

3. Nano-scaling of existing Chinese traditional medicines for diabetic foot ulcer (DFU) and Renal management as alternative therapeutics in an unexplored area and requires more attention [151, 156].
4. In the context of diagnostic procedures, there is a recognized need in regions for early diagnosis and treatment of sepsis. The field of magnetic nanosystems, in conjugation with photodynamic treatment, has demonstrated promising strategies and superior imaging capabilities [152]. Furthermore, imaging techniques, such as MRI, require optimization for the distribution and clearance capability of nanomedicine. Additionally, safety considerations and regulatory guidelines need to be upgraded for nanoparticles before they can be proposed for clinical trials, labeling/tagging, and testing [152].
5. In the context of cancer management, hepatocellular carcinoma (HCC), there is a need for research into targeting models that can be suitable and applicable to broad populations. Additionally, combinational therapies for HCC show greater potential compared to conventional single nanoparticle (NP) therapies due to their robust and effective targeting properties [4, 157]. In the case of colon cancer, formulation of nano drug with effective drug accumulation in lesions is the next objective, rather than settling in the non-targeted healthy tissues. The formulated drug should be capable of passing the first stage of hepatic metabolism [158]. To conclude, rapid development of methods for cancer detection and quantification at its primary stage is necessary [159].
6. Regarding other strategies, in multifunctional nanosystems, a simple, practically acceptable, and clinically applicable model should be the focus of future studies [106]. Effective mucolytics and integration of gene-editing tools can be incorporated into the treatment of diseases similar to cystic fibrosis [160].

A vital consideration is how these advanced nanomedicines affect the environment due to their biotoxicity, bioaccumulation, and environmental transformation. Assessments of risks associated with nanomedicines on human health must also be accomplished [137]. Thus, nanotechnology is a multidisciplinary field that extends many scientific disciplines, including chemistry, biology, physiology, material technology, and electronics. Therefore, cooperation among domains is necessary to ensure that advancements can result in useful products [133].

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Theranostic Potential of Nanomaterials in Neurodegenerative Diseases: Insights into Biosensing, Drug Delivery and Tissue Engineering

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1 Introduction

Neurodegenerative diseases (NDDs) comprise a range of disorders affecting the neural system, from acute central nervous system (CNS) injuries to chronic conditions. NDDs commonly include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and Huntington's disease (HD) [1]. NDDs present a significant challenge in biomedical research, especially as they become more prevalent among the elderly population and show poor responsiveness to treatment. Traditionally, treatment strategies for NDDs have focused on delaying neuronal death by inhibiting the formation of neurotoxins and modulating brain cell inflammation [2]. Despite extensive research efforts over the past decades, effective therapies for NDDs remain elusive, significantly impacting patients. Nonetheless, there is some hope in slowing down neurodegenerative processes through methods such as delivering anti-inflammatory drugs to the brain or utilizing cellular approaches like stem cell interventions [3]. NDDs contribute significantly to morbidity and premature mortality among aging populations worldwide, progressively impairing CNS function. While presenting with diverse clinical manifestations, most NDDs share a common pathology characterized by the accumulation of

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disease-specific proteins into insoluble aggregates [4, 5]. Additional pathogenic factors include mitochondrial dysfunction, misfolded protein aggregation, oxidative stress, neuroinflammation, ion accumulation, and excitotoxicity [6]. However, conducting clinical trials for CNS drugs is challenging due to the complexity of the brain, potential side effects, and the impermeability of the blood-brain barrier (BBB). These challenges underscore the need for innovative approaches to drug delivery to the brain, presenting new strategies and opportunities [7]. In recent years, theranostics has emerged as a highly beneficial approach to drug delivery. Coined to describe efforts in clinics aimed at developing detailed, personalized therapies for various diseases, theranostics integrates diagnostic and therapeutic capabilities into a single framework [8]. This approach can be implemented in two primary ways: first, diagnosis preceding therapy, enabling tailored treatment based on disease type identification; second, therapy following diagnosis, facilitating the identification of effective treatments for individual patients [9]. Nanotechnology has become pivotal in theranostic progress, encompassing the application of nanoscience to utilize nanomaterials and nano-sized components across diverse products. This field enables tailored material and product design, yielding novel properties [10]. Nanomaterials, renowned for their multifunctionality, offer benefits such as enhanced strength, lightweight construction, and heightened sensing and actuating abilities, amplifying their utility in various applications [11]. Various nanomaterials, including carbon-based nanomaterials, polymeric, and inorganic types, offer distinct advantages in different fields. Nanomaterials like carbon-based nanomaterials, liposomes, and micelles enable advancements in drug delivery, sensors, and nanoelectronics due to their versatility and biocompatibility [12]. Inorganic counterparts such as metal nanoparticles find applications in electronics, catalysis, and medicine owing to their small size and large surface area. Polymeric nanomaterials like nano polymers provide customizable structures crucial for drug delivery, tissue engineering, and coatings, contributing to innovation across industries [13]. In recent years, biomedical applications of nanomaterials have surged, revolutionizing drug delivery, biosensing, and tissue engineering. These advancements tackle classical challenges, enhancing precision and efficacy in diagnostics and therapeutics, thus offering promising avenues for innovation in diverse biomedical fields [14] (Fig. 1). In this chapter, we will delve into the theranostic applications of nanomaterials in the management of NDDs. We will explore how these advanced materials play a pivotal role in both diagnosis and therapy, offering targeted and effective approaches to mitigate the complexities associated with NDDs.

2 Neurodegenerative Diseases: Theranostic Challenges and Complications

NDDs represent a range of persistent and progressive neural conditions characterized by the gradual breakdown of neurons in specific regions of both the CNS and peripheral nervous system (PNS). This gradual neuronal deterioration leads to deficits in various brain functions, such as memory, motor abilities, and cognitive skills,

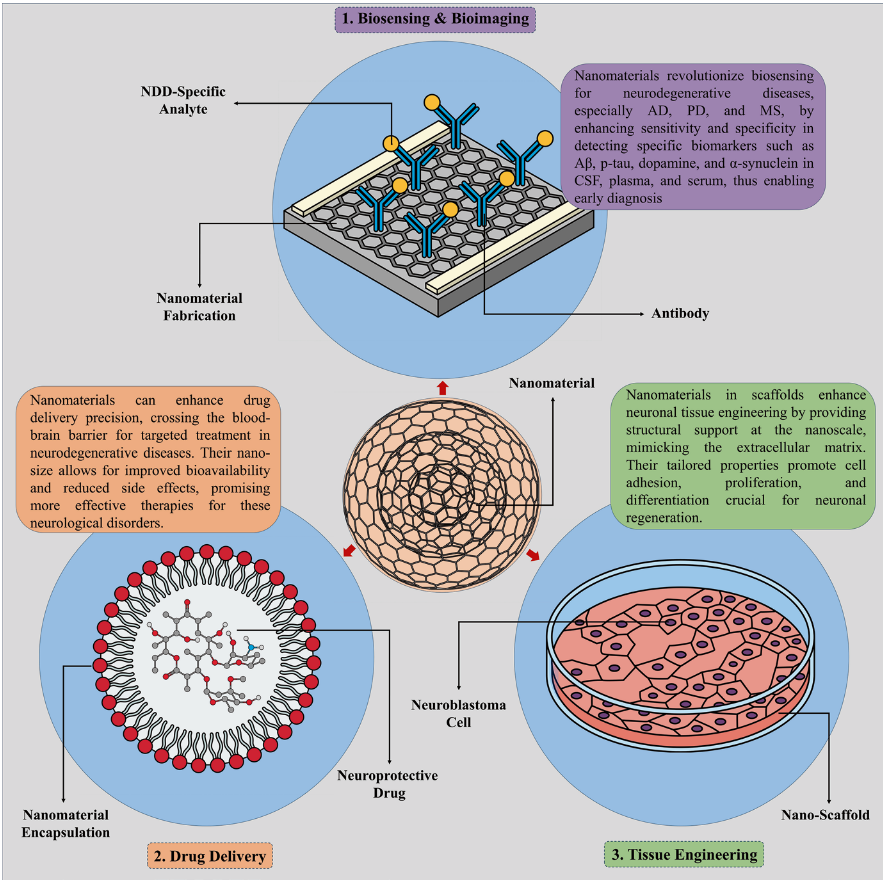
1. Biosensing & Bioimaging
NDD-Specific Analyte
Nanomaterials revolutionize biosensing for neurodegenerative diseases, especially AD, PD, and MS, by enhancing sensitivity and specificity in detecting specific biomarkers such as Aβ, p-tau, dopamine, and α-synuclein in CSF, plasma, and serum, thus enabling early diagnosis
Nanomaterial Fabrication
Antibody
Nanomaterial
Nanomaterials can enhance drug delivery precision, crossing the blood-brain barrier for targeted treatment in neurodegenerative diseases. Their nano-size allows for improved bioavailability and reduced side effects, promising more effective therapies for these neurological disorders.
Nanomaterials in scaffolds enhance neuronal tissue engineering by providing structural support at the nanoscale, mimicking the extracellular matrix. Their tailored properties promote cell adhesion, proliferation, and differentiation crucial for neuronal regeneration.
Neuroblastoma Cell
Neuroprotective Drug
Nanomaterial Encapsulation
Nano-Scaffold
2. Drug Delivery
3. Tissue Engineering

Fig. 1 Role of nanomaterials in biosensing, drug delivery, and tissue engineering in NDDs: Nanomaterials play crucial roles in biosensing by enhancing sensitivity and selectivity through their high surface area and tailored properties in NDDs. In drug delivery, they enable targeted and controlled release of neuroprotective medicines, improving efficacy while minimizing side effects. Moreover, in tissue engineering, nanomaterials contribute to scaffold design, promoting cell adhesion, growth, and differentiation for enhanced neuronal tissue regeneration. Overall, nanomaterials revolutionize biomedical applications by offering precise control and functionality at the nanoscale level in NDDs

all of which are governed by the affected regions of the CNS [15]. NDDs affect around 44 million people worldwide, predominantly in the later phases of life, such as those above 60 years of age. Regrettably, there are currently no successful treatments to halt or slow down the progression of neurodegeneration. Projections indicate a significant rise in cases to approximately 135 million globally by the middle of the century, marking a substantial increase from the current 44 million cases [16, 17]. Consequently, NDDs can disrupt the typical structure, function, or both of the nervous system, leading to a wide array of symptoms and impairments. Furthermore, these disorders can manifest in various forms, affecting sensory, motor, cognitive, or autonomic functions, with the severity varying greatly based on the specific condition and individual circumstances [18]. The pathophysiology of these diseases varies considerably, encompassing differences in pathological proteins, primary target cells, modes of action, and propagation within nervous tissue. These disorders can have diverse origins, including genetic predisposition, pathological infections, autoimmune reactions, degenerative processes, metastatic tumors, traumatic injuries, and vascular complications [19, 20]. For instance, in AD, subtle progression precedes cognitive impairment driven by neuronal, microglial, and astroglial alterations. Pathological factors encompass neuroinflammation, vascular modifications, and dysfunction in the lymphatic system. Aβ triggers the propagation of tau pathology, linked with necroptosis markers in neurons exhibiting granulovacuolar degeneration [21, 22]. In contrast, PD pathophysiology involves intricate interactions, including abnormal α-synuclein aggregation, mitochondrial and lysosomal dysfunction, issues in vesicle transport, synaptic disturbances, and neuroinflammation. These mechanisms collectively hasten the demise of predominantly dopaminergic neurons, impacting both motor and non-motor circuits [23, 24]. MS initiates with inflammatory demyelination, triggering a cascade that activates microglia, induces chronic oxidative injury, accumulates mitochondrial damage in axons, and promotes iron accumulation in the brain due to aging. Alterations in mitochondrial function, particularly in axons, play a pivotal role, generating chronic cell stress and disrupting ionic balance, ultimately leading to axonal and neuronal demise [25, 26]. HD demonstrates highly selective neuropathological changes, characterized by significant cell loss and atrophy in the caudate and putamen. Striatal medium spiny neurons, especially those containing enkephalin and projecting towards the external globus pallidum, are notably more vulnerable than neurons containing substance P and projecting towards the internal globus pallidum [27, 28].

Identifying clinically relevant biomarkers for neurological diseases presents distinct hurdles. Four primary challenges include: (i) limited tissue accessibility at the pathology site; (ii) inadequate clinical diagnostics and disease progression assessment at diagnosis; (iii) brain complexity and tissue heterogeneity; and (iv) insufficient functional endpoints and validation models [29]. Over the past two decades, research on nanomaterials like carbon nanotubes, graphene, polymeric, and inorganic nanowires and nanoparticles has surged, offering unique properties across electrical, mechanical, and optical domains. Leveraging these distinctions holds promise for diverse applications across sectors, with particular potential in sensor technology [30]. Biosensor devices utilize biological receptors to detect target analytes like DNAs, proteins, or molecules such as glucose or pollutants. Transducers convert biological interactions into measurable signals, like electrochemical or optical signals. Nanomaterials enhance biosensor sensitivity and lower detection limits by facilitating increased immobilization of receptors and acting as transduction elements [31]. Modified nanomaterials detect biomarkers of NDDs like Aβ, acetylcholine, dopamine, and α-synuclein. Biosensors are categorized by biological recognition elements and transduction principles; immunosensors and DNA sensors rely on antibody-antigen or DNA interactions. Improving surface area, chemical activity, and conductivity enhances biosensor sensitivity [14].

While there are drugs on the market that alleviate symptoms of NDDs, a definitive drug capable of reversing neurodegeneration remains elusive in these diseases to date. The majority of these diseases lack specific, effective therapies due to the challenge of drug delivery across the BBB to reach the brain in sufficient quantities for pharmacological impact [32]. The BBB presents a significant obstacle, with approximately 95% of drugs unable to traverse it due to their molecular properties. This barrier, along with the blood-cerebrospinal fluid barrier, restricts access to the CNS. Lipophilic drugs with a molecular weight of less than 500 Da can penetrate the BBB, whereas conventional drugs often fail to meet this criterion [13]. The BBB's semipermeable nature limits the entry of therapeutic agents into the CNS, posing a significant challenge to modern medicine. Alternative approaches such as photothermal and photodynamic therapies have been explored for treating neurological disorders by bypassing the BBB, yet they carry risks including tissue damage and photosensitization [33]. Consequently, there is an urgent demand for the development of therapeutic agents capable of crossing the BBB without adverse effects to address these diseases. However, achieving this goal necessitates a profound understanding of the mechanisms and underlying causes of each disease for effective treatment [13]. The development of therapeutic strategies capable of traversing the BBB while enhancing efficacy is imperative. Continual research efforts are dedicated to devising delivery mechanisms for therapeutics to address this challenge. Various strategies have emerged to tackle this obstacle, with the nano-based approach leading to the forefront of advancement in therapeutic delivery [34, 35] . Nanotechnology leverages engineered nanomaterials and nanoparticles, typically ranging from 1 to 100 nm in at least one dimension, to navigate these challenges. In the realm of biomedical science, nanotechnology, and nanomaterials offer promising avenues, with diverse nanoparticles like carbon nanomaterials, polymeric nanoparticles, and inorganic nanoparticles being employed in brain studies and research [12].

In addition, tackling NDDs through tissue engineering poses formidable challenges owing to the intricate structure of neural tissue and the complexities inherent in stimulating regeneration [36]. These hurdles stem from various factors, notably the demand for meticulous control over scaffold fabrication, encompassing adjustable topography and biochemical signals. Neural tissue engineering endeavors to mend and rejuvenate impaired neural systems, a task particularly daunting in afflictions such as AD, PD, MS, and others [37]. A primary impediment lies in the multifaceted nature of neural injuries, encompassing both primary and secondary damage mechanisms culminating in transient or enduring neurological impairments [38]. The inflammatory cascade triggered by such injuries can impede nerve regeneration and worsen the damage, underscoring the imperative to address inflammation and fibrosis upon introducing foreign substances in tissue engineering methodologies [39]. Nanomaterials are essential in advancing neuronal tissue engineering, offering unique properties like exceptional conductivity, flexibility, and the ability to accelerate neuronal cell growth [40]. Notably, carbon nanosheets and polymeric nanomaterials are promising in facilitating cell adhesion and proliferation and encouraging neuronal cell differentiation, thereby enhancing neuron regeneration in NDDs like AD, PD, and MS [41] (Fig. 1).

3 Nanomaterials: An Alternative to Available Conventional Theranostics

A nanomaterial is defined as a substance with organized elements at the nanometer scale (usually between 1 and 100 nm) or demonstrates properties influenced by its nanoscale arrangement. These materials frequently showcase distinctive physical, chemical, and biological traits owing to their small size, shape, surface area, and other nano-level attributes [42]. Through deliberate design, nanomaterials can attain remarkably expansive surface areas. These materials can exhibit exceptional magnetic, electrical, optical, mechanical, and catalytic characteristics, markedly distinct from their bulk forms. By finely adjusting parameters such as size, shape, synthesis conditions, and appropriate functionalization, nanomaterial properties can be tailored as needed for applications in biomedical theranostics [43]. Nanotheranostics stands as a monumental scientific advancement within nanomedicine. Present diagnostic and therapeutic methods often entail invasiveness, prolonged procedures, and harsh side effects. However, the emergence of nanotheranostics offers a promising solution, leveraging nanotechnology and nanomaterials to integrate diagnostics and therapeutics [11, 44].

3.1 Carbon-Based Nanomaterials: Properties, Synthesis, and Applications

Carbon-based nanomaterials, including graphene, carbon nanotubes (CNTs) and fullerenes exhibit diverse properties and applications ranging from drug delivery to electronics, owing to their unique structures and functionalities. In subsequent sections, we will extensively cover carbon-based nanomaterials such as graphene, fullerenes, and CNTs primarily utilized in biomedical applications [45]. Graphene, composed of a single layer of carbon atoms arranged in a honeycomb lattice, forms a remarkable two-dimensional planar structure. Its unique atomic arrangement grants graphene exceptional mechanical, electrical, and thermal properties, making it a versatile material with diverse applications in fields such as electronics, materials science, and biotechnology [46]. Graphene stands out as a highly desirable functional nanomaterial, prized for its exceptional attributes including outstanding tensile strength, electrical conductivity, carrier mobility, elasticity, thermal conductivity, and remarkable optical transparency of approximately 97%. Additionally, its lightweight nature further enhances its appeal across various fields of application [9]. Because of its remarkable characteristics, graphene finds widespread application across diverse fields including electrochemiluminescence (ECL) sensors, water filtration, energy storage devices, biosensors, transistors, solar cells, drug delivery systems, tissue engineering, multi-model imaging, and photothermal therapy [47]. Graphene oxide (GO), a derivative of graphene formed through graphite oxidation, incorporates oxygen functional groups. With high hydrophilicity, it readily disperses in water, boasting impressive properties including elasticity, tensile strength, electrical conductivity, thermal stability, and cost-effectiveness [48]. CNTs are cylindrical structures formed from rolled-up graphene sheets. They exist in two primary forms: single-walled carbon nanotubes, with diameters as small as 0.7 nm, and multi-walled carbon nanotubes, which can reach diameters of up to 100 nm [46]. CNTs possess exceptional tensile strength and elasticity, making them robust materials. Additionally, they exhibit excellent thermal conductivity properties. Even under high forces, CNTs can undergo binding, twisting, kinking, and buckling without sustaining damage, showcasing their remarkable structural resilience [49]. Nanotubes find diverse applications in nanotechnology, including high-strength composites, nanoprobes, sensors, and various medical fields. However, this discussion delves specifically into their biomedical applications such as tissue engineering, cancer cell identification, and drug and gene delivery [50]. Three key challenges must be overcome for the widespread use of carbon nanotubes in biological and medical contexts: addressing their toxicity, pharmacology, and functionalization. A significant obstacle lies in the insolubility of carbon nanotubes in aqueous solutions, prompting researchers to modify their surface through functionalization with hydrophilic compounds and chemistries to enhance water solubility [51]. Fullerene exhibits a closed, hollow cage-like structure with interconnected carbon atoms forming pentagonal and hexagonal rings. Its inherent hydrophobic nature necessitates enhancement of water solubility through the addition of carboxyl, amino, or hydroxyl groups, thereby improving its biocompatibility [45]. Fullerenes exhibit versatile therapeutic potential, encompassing antiviral, antioxidant, and neuroprotective activities, along with applications in drug and gene therapy, X-ray contrast imaging, antimicrobial functions, and as carriers for drug delivery and preservation. Their efficacy stems from attributes like high electrochemical stability, small size, specific morphology, well-ordered structure, high electroconductivity, excellent thermal conductivity, and unique mechanical properties [52].

Various methods exist for nanomaterial synthesis, with top-down and bottom-up approaches being predominant. Top-down techniques include lithography, mechanical milling, sputtering, laser ablation, electron explosion arc discharge, and thermal decomposition. Bottom-up methods encompass chemical vapor deposition (CVD), sol-gel, spinning, pyrolysis, and biological synthesis. For instance, creating carbon nanotubes can be achieved *via* techniques such as CVD, laser ablation, and carbon arc discharge [53]. Graphene synthesis employs diverse methods. Top-down approaches involve breaking down precursors like graphite to form graphene sheets, utilizing techniques such as mechanical exfoliation, oxidation, liquid phase exfoliation, and GO reduction. Bottom-up methods involve synthesizing graphene on substrates, utilizing techniques like chemical vapor deposition, epitaxial growth, and total organic synthesis, often employing carbon source gases [54]. Recent advancements in nanotechnology have significantly enhanced diagnostic and treatment approaches, with carbon-based nanomaterials emerging as key contributors. Carbon-based nanomaterials, such as CNT and graphene, exhibit considerable promise in the realm of theranostics for different diseases, including NDDs [14]. These versatile nanomaterials serve as multifunctional platforms for targeted drug delivery, tissue engineering, regenerative medicine, biosensing, and diagnostic imaging. Their unique physicochemical properties facilitate accurate identification of particular biomarkers, precise delivery of targeted drugs, and promotion of tissue regeneration for advancements in regenerative medicine [14].

3.2 Polymeric Nanomaterials: Properties, Synthesis, and Applications

Polymeric nanomaterials are composed mainly of polymer molecules, intentionally designed to be within the nanoscale range, typically 1–100 nm. These materials are constructed from polymers, which are large molecules made of repeating subunits. Polymer-based nanoparticles are macromolecules formed by linking monomers together to create polymer chains [55]. Polymeric nanoparticles such as PLGA (poly(lactic-co-glycolic acid)), PCL (polycaprolactone), and chitosan (CS) nanoparticles are widely used for drug delivery due to their biocompatibility and tunable properties. They have the capability to encapsulate drugs, proteins, or genes, shielding them from degradation and facilitating precise release at specific locations in the body [56]. Their prominence stems from several advantageous characteristics, including the potential for varied compositions, straightforward synthesis methods, facile functionalization, and variances in surface chemistry, achievable through adjustments to the base polymers to suit specific applications [57]. A polymeric nanomaterial comprises three key elements: (1) a polymeric component ensuring solubility and biocompatibility, (2) a chemical component customized for imaging purposes, and (3) a therapeutic component designed for drug or gene transport [58]. Polymeric nano-platforms offer several advantages in theranostics, including: (1) controllable particle size and degradation rate, (2) low toxicity at minimal concentrations, (3) spaciousness for cargo encapsulation due to their porous structure, (4) ease of surface modifications with diverse target molecules, and (5) biodegradation into non-toxic materials [56, 59]. Polymeric nanoparticles have the added benefit of undergoing degradation and elimination within the human body without triggering immune responses. This is attributed to the fact that most monomers composing these nanoparticles naturally occur in the body. As a result, they are generally well-tolerated and can be safely metabolized or excreted. This feature enhances the biocompatibility of polymeric nanomaterials, making them suitable for various biomedical applications [60].

PLGA nanomaterials is a biodegradable, hydrophobic copolymer and mainly composed of two monomers bonded with ester linkage: lactic acid and glycolic acid, which also form synthetic biodegradable polymers of Poly(lactic acid) (PLA) and Poly(glycolic acid) (PGA) nanomaterials respectively. PLGA is widely adapted for preparing nanoparticles encapsulating hydrophobic and hydrophilic anti-therapeutic agents because of hydrophobic and hydrophilic nature of PLA and PGA, respectively [61]. PLA and PGA exhibit differences primarily due to the side methyl group in PLA, reducing crystallinity and increasing hydrophobicity. Modulating the PLA to PGA ratio allows precise control over the properties of PLGA, making it versatile for various biomedical applications. Adjusting this ratio offers tunability in degradation rates, mechanical strength, and drug release kinetics of PLGA-based materials [55]. PLGA undergoes functionalization with poly(ethylene glycol) (PEG) to create diverse block copolymers of polyesters with PEG. The inclusion of a PEG layer serves as a barrier, mitigating interactions with foreign molecules through steric and hydrated repulsion mechanisms. This modification enhances the biocompatibility and stability of PLGA-based materials, making them suitable for various biomedical applications [62, 63]. PLGA is approved by the European Medicine Agency and the US Food and Drug Administration as an ideal material for designing a drug delivery system due to its outstanding biocompatibility and biodegradability [64]. Achieving the desired shape and size of polymeric nanoparticles involves employing two major methodologies: top-down and bottom-up strategies. Top-down methods, such as emulsification and double emulsion approaches, often use organic solvents, but injection-based methods can mitigate their disadvantages. Bottom-up methods, like coacervation, inclusion complexation, and nanoprecipitation, offer alternative approaches for nanoparticle synthesis [65]. Regardless of the nanoparticle formation method, a drying technique is commonly necessary to remove solvent, minimizing polymer hydrolysis and drug leakage risks. This process ensures the stability and integrity of the nanoparticles, enhancing their efficacy and safety for various biomedical applications [55]. Biodegradable nanomaterials offer a promising avenue for controlled release systems, owing to their ability to be metabolized harmlessly. This biocompatibility enables site-specific drug targeting, enhancing therapeutic efficacy while minimizing adverse effects. Notably, polymeric nanomaterials such as PLGA, PLA, and CS have been extensively employed in these applications due to their favorable properties [66, 67]. They can cross the BBB, effectively control the concentration of the drug over time, and release the active molecule at the site of action. All these features contribute to their effectiveness in drug delivery systems. Polymeric nanomaterials have also been utilized in biomarker detection for NDDs, depending on the imaging technique used [68].

3.3 Inorganic Nanomaterials: Properties, Synthesis, and Applications

Inorganic nanomaterials have emerged as a promising area of research and development due to their unique properties and wide range of applications. These Nanomaterial which are typically composed of metals, metal oxide, or metal-based compounds, exhibit size-dependent properties that differ from their bulk counterparts. Inorganic nanoparticles encompass a variety of materials including iron, gold, silica, and zinc [69]. One prominent example is iron oxide nanoparticles (IONPs), which consist of ferromagnetic materials and exhibit exceptional magnetic properties. IONPs, found in magnetite or hematite forms, can be tailored for diverse applications by surface modification with various inorganic molecules and ligands [70]. Due to their distinct magnetic properties, nontoxicity, and biodegradability, IONPs have found increasing use in magnetic resonance imaging (MRI), cell separation, targeted drug delivery, and therapy. They play a crucial role in cancer treatment, enhancing the efficacy of chemotherapeutic drugs in combination therapy [71]. The distinctive features of IONPs, including high field irreversibility, super magnetism, and anisotropic contributions, render them exceptionally remarkable in various applications. Particularly in disease monitoring, IONPs are primarily absorbed by macrophages, providing a valuable tool for tracking these immune cells in disease condition [72]. However, despite their numerous advantages, there are drawbacks to consider. For instance, IONS necessitate an external surfactant coating to prevent particle aggregation, and this coating is essential to prevent disintegration since naked nanoparticles are generally not biocompatible and could release toxic by-products [73]. In addition, gold nanoparticles (AuNPs) are widely utilized in bio-applications due to their surface modifiability with polymers, drugs, antibodies, and proteins. They possess exceptional light absorption properties, enabling optical detection of various antigens and targeted drug delivery. AuNPs excel in biological imaging, particularly in light interaction, making them pivotal in various biomedical techniques [74, 75]. AuNPs are essential in stem cell therapy for visualizing cell proliferation and tissue integration, leveraging their optical properties. In addition, these AuNPs have shown anti-inflammatory and antioxidant characteristics and are also effective scavengers of ROS, improving the activity of antioxidant enzymes [76, 77]. However, unmodified AuNPs face challenges in stability and targeting capability for *in vivo* applications. These limitations are addressed through surface modifications with PEGs and glycol chitosan (GC) to enhance stability [78]. Apart from AuNPs, silver nanoparticles (AgNPs) are pivotal in disease diagnosis and therapy, boasting diverse applications and therapeutic potential, notably in cancer treatment. Their antimicrobial, antibacterial, antioxidant, and anti-inflammatory properties make them promising candidates for addressing various medical challenges, including drug solubility and systemic delivery issues in cancer therapy [79]. Silica nanoparticles offer ideal traits for clinical use: precise shape and size control, low toxicity, superb biocompatibility, stability in body conditions, and safe degradation products like orthosilicic acid excretable by the kidneys. Mesoporous silica nanoparticles (MSNPs) facilitate efficient transport of active agents *via* their porous matrix, utilizing physical or chemical bindings [80]. While silica is an essential nutrients for humans in small doses, prolonged exposure to non-PEGylated silica nanoparticles at concentrations exceeding 50 mg/L for durations surpassing 48 hours has been shown to be toxic in in vitro conditions [81]. Surface modification of MSNPs involves functionalization with polyethyleneimine polymer, enabling efficient delivery of molecules like DNA and siRNA. This modification also creates a cationic surface, facilitating the attachment of these molecules for targeted delivery systems [82]. There are various methods involved in the synthesis of these NP. To synthesize AuNPs, generally colloidal methods is utilized to synthesize AuNPs structures such as spheres, nanorods, and nano-cages, another galvanic replacement methods for hollow AuNPs [83]. Fe_3O_4 nanoparticles, a superparamagnetic material, are synthesized *via* thermal decomposition, co-precipitation, or hydrothermal methods. These methods offer precise control over size and morphology, vital for various applications. Their unique properties make them promising candidates in biomedical, environmental, and technological fields [84]. These inorganic nanomaterials exhibit promise in NDDs for diagnostics and therapeutics, owing to their adaptable surface chemistry and BBB-penetrating capabilities. Their tunable properties make them viable for targeted drug delivery and imaging, suggesting a significant advancement in NDD treatments [85]. Enhanced optical and magnetic properties of the material offer avenues for non-invasive imaging. This facilitates the monitoring of disease progression within the central nervous system. Such advancements hold promise for improved diagnostics and treatment monitoring [86].

4 Nanomaterials in the Theranostics of Neurodegenerative Diseases (NDDs)

Nanomaterials are pivotal in theranostics for NDDs, enabling targeted drug delivery and diagnostics with unique properties enhancing therapeutic efficacy, reducing toxicity, ensuring stability, and facilitating precision [87]. Graphene, metallic and polymeric nanoparticles, carbon nanotubes, liposomes, and micelles are extensively employed, crossing the BBB for precise imaging and treating conditions like AD, PD, MS etc. These materials offer promise in revolutionizing drug delivery strategies and addressing challenges in NDDs [13, 88]. In addition, nanomaterials provide sensitive detection of neuronal markers, leveraging their high surface area-to-volume ratio and tunable chemistry for enhanced selectivity. Integrated into biosensing platforms, they facilitate the development of precise diagnostic tools, advancing early detection and personalized treatments for NDDs [89] (Fig. 1).

4.1 Role of Carbon-Based Nanomaterials in Neurodegenerative Diseases (NDDs)

Nanomaterials, distinguished by their remarkably diminutive size, hold promise across various industrial, biomedical, and electronic domains. Carbon-based nanomaterials exhibit exceptional physicochemical traits, including chemical stability, high conductivity, remarkable photoluminescence, and easy functionalization. These properties render them highly promising for diverse biomedical applications such as drug delivery, diagnostics, imaging, and therapy [90] (Table 1).

Table 1 Role of Carbon-based, polymeric, and inorganic nanomaterials in biosensing, drug delivery, and tissue engineering in different NDDs

Application	Types of NMs	NMs	Experimental Findings	Reference
Biosensing & Bioimaging	Carbon Nanomaterials (C-NMs)	Graphene	Graphene-based nanobiosensors have been extensively investigated recently for Aβ and Tau, to identify the prospective biomarkers of AD	[92]
			Surfaces coated with oxygen-plasma-treated rGO were utilized as reactive interfaces to identify Aβ peptides, which are pathological indicators linked to AD.	[93]
			Reduced GO-based acetylcholinesterase (AchE) modified field-effect transistor (FET) biosensor for monitoring drug effects in AD treatment.	[94]
			A GO-based electrochemical sensor for the detection of serum Tau-441, a protein biomarker for AD was recently developed.	[95]
			A label-free biosensor for the identification of critical AD biomarkers was developed using electrochemically reduced GO (erGO) and a highly conductive dual-layer graphene.	[96]
			The primary biomarkers of PD, dopamine, and α-synuclein, have been widely identified using graphene-based nano-biosensors in artificial serum.	[97]
			A novel rGO nanocomposite coated with MnS nanoparticles exhibited exceptional electrochemical sensing capabilities for PD diagnosis, detecting dopamine at low nanomolar levels.	[98]
			GO and thionine via electrostatic interactions, a biosensing platform was formed for enabling sensitive ratiometric electrochemical detection of uric acid.	[14]
	Polymeric Nanomaterials (P-NMs)	Chitosan	Chitosan (CS) and hyaluronic acid nanoparticles cross-linked with glutaraldehyde functioned as a theranostic agent for both in vitro and in vivo imaging/probing and Aβ fibrillization suppression.	[127]
			a U-shaped fiber optic probe, immobilized with AuNP and functionalized with a CS film, served as a sensing platform for α-synuclein detection.	[128]
			A sensor was developed using a glassy carbon electrode modified with rGO, carbon black, and a CS film for the detection of dopamine.	[129]
		PLA	PLA-graphene electrodes (PLA-G), including increased surface area, electron transfer rate, and edge site quantity for dopamine.	[130]
		PLGA	Novel magnetic beads with enhanced antibody capture for Tau protein were developed using PLGA-Zinc matrix.	[68]

	Inorganic Nanomaterials (I-NMs)	Au-NPs	An interdigitated microelectrodes as an impedance biosensor for blood-based Aβ detection was developed using AuNPs sandwich assay.	[156]
			An AuNP-polyglutamic acid (PGA)-modified indium tin oxide (ITO)-based disposable neuro-biosensor system was developed for the detection of CSF α-synuclein	[157]
		Ag-NPs	A novel approach for detecting dopamine using a silver nanoparticle (AgNP) modified electrode coated with GO was developed.	[158]
		Fe-NPs	The Fe-based MOF-armored HRP addressed thermal instability, enabling successful Aβ oligomer measurement in blood serum samples.	[159]
			A novel method was employed to fabricate α-Fe_2O_3@erGO nanocomposite, and a modified sensor was created through direct electrochemical reduction for the detection of dopamine.	[160]
		TiO_2-NPs	The TiO_2-NB PEC sensor was developed that demonstrated high sensitivity and low limit of detection for detecting a wide range of $A\beta_{1-28}$ peptides.	[161]
			A highly sensitive sensor utilizing electrospun TiO_2 nanofiber/graphite oxide paste/GCE was developed for voltammetric determination of levodopa (L-DOPA).	[162]
			A highly sensitive impedimetric immunosensor using TiO_2 for detecting Anti-Myelin Basic Protein (Anti-MBP), an autoantibody associated with MS, was developed.	[163]

(continued)

Table 1 (continued)

Application	Types of NMs	NMs	Experimental Findings	Reference
Targeted Drug Delivery	Carbon Nanomaterials (C-NMs)	Graphene	Graphene oxide (GO) loaded with dauricine protects against Aβ_{1-42}-induced oxidative damage and apoptosis in both *in vitro* and *in vivo* AD models.	[105]
			GO loaded with rivastigmine is an excellent drug entrapment tool with high efficiency and controlled release in AD treatment.	[106]
			GO loaded with puerarin has an excellent drug-loading ability, modifiable surface functional groups, and good biocompatibility for the treatment of PD.	[108]
			Polyethylene glycol (PEG) was used to conjugate nGO, and the resulting PEG-nGO was functionalized with the MS medication FTY720 *via* an amide bond to create drug conjugated.	[109]
			Ni-doped graphene nanosheet functions as a prominent nano-carrier of cladribine for the treatment of MS.	[110]
	Polymeric Nanomaterials (P-NMs)	PLC	Investigation of polycaprolactone (PLC) nanocapsules carrying 7-Methoxytacrine (7-MEOTA) as an approved treatment for AD, showed better results.	[138]
		Chitosan	CS-functionalized PLGA core/shell nanoparticles (CH@Cur-PLGA C/S NPs) carrying curcumin was developed for intranasal delivery for AD therapy	[134]
			To boost Astragaloside IV's (ASI) efficacy in MS, β-asarone modified ASI-loaded CS nanoparticles (ASI-βCS-NP) were tailored for nose-to-brain delivery.	[135]
			CS nanoparticles incorporating rivastigmine, an AD drug prepared *via* spontaneous emulsification, exhibited better therapeutic results.	[137]
	Inorganic Nanomaterials (I-NMs)	Fe-NPs	A superparamagnetic iron oxide nanocube (SPIONs)-based delivery system was developed to effectively deliver epigallocatechin gallate (EGCG) for inhibiting α-synuclein aggregation in PD treatment.	[181]
			A multifunctional conjugate was developed utilizing magnetite iron oxide nanoparticles (MIONPs) for cellular delivery of siRNA targeting BACE1 gene expression implicated in AD.	[165]
			A novel curcumin delivery system employing Fe_3O_4@carbon dots nanocomposite (Fe_3O_4@CDs) as the carrier has been developed for AD therapy.	[182]
		TiO_2-NPs	HEMA/PEG-DA-based hydrogels with TiO_2 nanoparticle was developed to release of Donepezil hydrochloride, an AD treatment drug.	[168]

Tissue Engineering & Regenerative Medicine	Carbon Nanomaterials (C-NMs)	Graphene	GO ameliorates the cognitive impairment through inhibiting PI3K/Akt/mTOR pathway to induce autophagy in AD mouse model	[118]
			PLCL-GO scaffold supports the alignment, growth, migration and differentiation of neural stem and progenitor cells without a need of a laminin coating for neuronal tissue engineering.	[184]
			GO nanosheets were applied as coatings on poly-L-lactide (PLLA) nanofibers, enhancing surface roughness to promote PC-12 neuronal adhesion and proliferation.	[121]
			GO-DMAEMA composite film extended the highest number of total outgrowth and branches of each neuron	[185]
			rGO/silk-based composite micro/nano-fibrous scaffolds regulate neuronal cell behavior in vitro for nerve regeneration and nerve tissue engineering.	[122]
			rGO-films were found to stop the loss of DA neurons and the drop in α-synuclein levels in an oxidative stress-induced PD model.	[119]
		Liposomes	Liposomal paclitaxel (PTX) holds promise for promoting axon regeneration in spinal cord injury repair when delivered with neural stem cells.	[114]
			The presence of liposomes modified hydrogel properties based on CS directly influence their capacity to differentiate PC12 neuronal cells.	[115]
		Micelles	Polymeric micelles based on multifunctionalized CS delivered pVGF to the brain, crucial for neurogenesis, learning, synaptic, and cognitive functions in AD.	[116]

(continued)

Table 1 (continued)

Application	Types of NMs	NMs	Experimental Findings	Reference
	Polymeric Nanomaterials (P-NMs)	PCL	Scaffolds using polyaniline, PCL, and gelatin has shown to enhance neural stem cell proliferation and facilitate neurite outgrowth.	[143]
		Chitosan	CS-gelatin-green tea extract composite particles enhance cell survival, reduce apoptosis, inhibit ROS production, elevate TH enzyme expression, and lower α-synuclein protein levels.	[147]
			Crosslinked nanofibers of CS and gelatin, mimicking the natural ECM, were developed for neural tissue engineering, exhibiting adequate biocompatibility in MS.	[148]
		PLGA	Unconjugated PLGA nanoparticles exhibit potential therapeutic benefits in both cellular and animal models of AD by promoting or inhibit the breakdown of Aβ aggregates.	[145]
			In 5xFAD mice, 28-day native PLGA treatment reduces Aβ plaque load and enhances cognitive function.	[146]
			PLGA microcarriers with collagen increased TH production confirms successful NT-3 overexpression, promoting dopamine neuron development.	[150]
			PLGA microfiber scaffolds with FAD iPSC-derived neurons facilitate neuronal development, increased survival, and decreased proliferation.	[144]
	Inorganic Nanomaterials (I-NMs)	Au-NPs	AuNP-decorated electrospun nanofiber scaffolds have been shown to promote extended neurite outgrowth.	[183]
			AuNPs enhanced survival of hNSCs treated with Aβ by decreasing caspase 3 and 9 activities, and mitochondrial dysfunction.	[172]
			PCL/PLGA film scaffolds, decorated with AuNP and IKVAV-pentapeptide, facilitated neural stem cell differentiation and axonal alignment.	[171]
		TiO_2-NPs	TiO_2 mitigated the formation of fibrils, presumably triggered by a photocatalytic mechanism, by diminishing the core structure of $A\beta_{1-42}$ in AD.	[174]
			TiO_2-nanowires alongside cerebrolysin (CBL), a compound containing active peptide fragments and neurotrophic factors, notably decreased Aβ deposits	[175]
		MnO_2-NPs	PEDOT/MnO_2 nanoellipsoids enhance neurite outgrowth and enable real-time monitoring of PC12 neuronal cells.	[177]
		Mn_2O_3-NPs	Mn_2O_3 exhibits enhanced inhibition of α-synuclein fibrillization and acts as ROS scavengers following cellular uptake via the caveolin-mediated pathway.	[13]

Role of Carbon-Based Nanomaterials in Biosensing in NDDs

Carbon-based nanomaterials that are used in biosensors encompass classic allotropes like amorphous carbon, graphite, and diamond, as well as newly identified allotropes like CNT, GO, graphene quantum dots, and fullerenes [91]. Graphene finds wide use in the biosensor field as it exhibits many significant properties, including optical transparency, mechanical flexibility, chemical stabillity, functionalization possibility, high conductivity, and charge carrier mobility. Other members of the graphene family are GO and reduced graphene oxide (rGO) [14]. GO has strong ionic interaction between the negatively charged -COOH group and the positively charged nuclear base. Furthermore, because of its hydrophilic nature, GO is easily dissolved in water, which facilitates handling and surface modification. Graphene-based nanobiosensors have been extensively investigated recently for Aβ and Tau, to identify the prospective biomarkers of AD [92]. For instance, surfaces coated with oxygen-plasma-treated rGO were utilized as reactive interfaces to identify Aβ peptides, which are pathological indicators linked to AD. This innovative diagnostic method demonstrates how oxygen-plasma treatment improves sensor performance without the need for complex fabrication techniques [93]. In a separate study, to study the enzymatic kinetics between acetylcholine and acetylcholinesterase (AChE), which are linked to the development and treatment of AD, an enzyme-modified field-effect transistor (rGO-EnFET) based on rGO was created. The accurate measurement of ACh quantification within the concentration range of 1 μM to 10 mM was assessed [94]. Additionally, a new GO-based electrochemical sensor for the detection of serum Tau-441, a protein biomarker for AD, is presented in a recent work. Using square wave voltammetry (SWV), the electrochemical sensor produced a linear response to Tau-441 with a LOD of 75fM in the concentration range of 0.08 pM-80pM [95]. Moreover, a label-free biosensor for the identification of critical AD biomarkers was developed using electrochemically reduced GO (erGO) and a highly conductive dual-layer graphene. Utilizing a wide linear range spanning from 11 pM to 55 nM to generate a low limit of detection (LOD) of 2.398 pM, the biosensor demonstrated exceptional selectivity against $A\beta_{1\text{-}40}$ and ApoE-4 [96]. In addition to AD, the primary biomarkers of PD, dopamine and α-synuclein, have been widely identified using graphene-based nano-biosensors. An impedance sensor intended to detect preclinical PD biomarkers was the focus of the first discovery. Utilizing only 30 μL sample per test, the sensor demonstrated a reliable linear response from 5pM to 1000pM, with a significant LOD at 1.2 pM, when used for the non-faradaic detection of α-synuclein autoantibodies inside the artificial serum [97]. In another study, Chen et al. recently presented a straightforward ultrasound-assisted method for creating a novel rGO nanocomposite coated with MnS nanoparticles. The α-MnS@rGO nanocomposite revealed exceptional electrochemical sensing capabilities for the diagnosis of PD, with a low nanomolar detection limit of 3.5 nM and a high linear dopamine range between 0.02 μM and 438.6 μM [98]. Similar to AD and PD, graphene-nanomaterials are also used to sense a few crucial biomolecules in MS. By self-assembling GO and thionine *via* electrostatic interactions, a biosensing platform was formed, and Au-Ag NPs/GO/thionine@

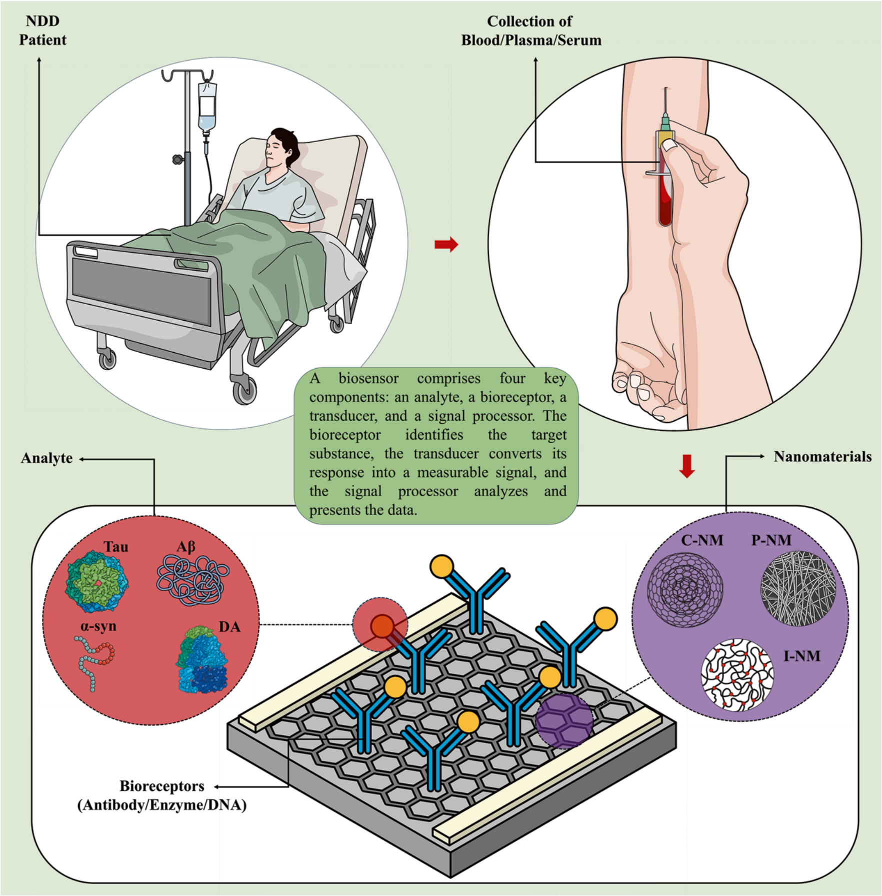
NDD
Patient
Collection of
Blood/Plasma/Serum
A biosensor comprises four key components: an analyte, a bioreceptor, a transducer, and a signal processor. The bioreceptor identifies the target substance, the transducer converts its response into a measurable signal, and the signal processor analyzes and presents the data.
Analyte
Nanomaterials
Tau
Aβ
α-syn
DA
C-NM
P-NM
I-NM
Bioreceptors
(Antibody/Enzyme/DNA)

Fig. 2 Role of different nanomaterials in biosensing disease-specific molecules in NDDs: Various nanomaterials play crucial roles in biosensing disease-specific molecules in NDDs, like AD, PD, MS, and HD. The nanomaterials, especially graphene, carbon nanotubes enhance electrical conductivity for sensitive detection. In addition, inorganic metal nanoparticles enable amplification of signals, improving detection limits. Moreover, the nanoporous materials provide large surface areas for enhanced binding interactions, enhancing sensitivity in disease biomarker detection for NDDs. Nanomaterial-based electrodes are capable of detecting various analytes, including Aβ, α-synuclein (α-syn), tau, dopamine (DA), using diverse bioreceptors such as analyte-specific antibodies, enzymes, or DNA in NDDs

GCE was the result of drop-coating onto Au-Ag bimetallic nanoparticles. This sensor achieved a low LOD of 0.3 µM, enabling sensitive ratiometric electrochemical detection of uric acid (UA) in human serum and urine [14]. In a different study, a micro-ball-fiber sensor probe was made with an advanced fusion-splicer, and by covering it with GO and AuNPs to detect uric acid, its sensitivity was raised. The constructed sensor showed good linearity slope, LOD of 65.60 µM, and sensitivity of 2.1%/mM [99] (Fig. 2).

Role of Carbon-Based Nanomaterials in Drug Delivery in NDDs

Nanotechnology provides the means to employ nanoparticles for illness prevention and health enhancement. The use of nanoparticles as drug carriers to improve the safety and pharmaco-efficacy of traditional medicines is of great interest. Because of their adjustable physiochemical and structural characteristics, nanoparticles can be used as medication carriers with a number of benefits over traditional treatments [100]. By altering the properties of the loaded medication in a way that supports the therapeutic goals, loading conventional therapies into nanoparticles, in conjunction with precise design and perfect tuning, can increase pharmacokinetic/pharmacodynamic properties, effectiveness, and solubility. Additionally by lowering organ toxicity and delivering the medication non-selectively to unwanted locations, these nanoformulations may further improve the drug's safety and acceptability [101]. A study revealed that organic nanoformulations like liposomes and PLGA nanoparticles *via* nasal administration, gaining traction for its direct brain access, showed promising results with liposomal rivastigmine. It exhibited superior pharmacokinetic parameters including rapid onset, higher C_{max}, enhanced bioavailability, prolonged half-life, and reduced clearance compared to oral administration in AD [102]. In a recent study, the liposomal system was shown to effectively deliver dopamine across the BBB to the brain, as demonstrated in a mouse model of PD. It resulted in a sustained increase in striatal dopamine levels for at least 3 hours post-administration, suggesting slow release. This promising delivery system, facilitating compound penetration through the BBB, holds potential for treating PD and other brain diseases [103]. Carbon-based nanomaterials such as GO, fullerenes *etc.* help deliver peptides, big proteins, and small-molecule medications because of their ultrathin, layered structure and high surface-to-volume ratio. High therapeutic loading and sustained drug release are made possible by their huge surface area [104]. It was recently shown that a dauricine-loaded GO nanoformulation showed anti-inflammatory and anti-oxidative stress characteristics in addition to preventing Aβ peptide misfolding and aggregation. In mice with $A\beta_{1\text{-}42}$-induced AD, it markedly improved cognitive memory deficits and greatly decreased brain glial cell activation [105]. In a different study, a novel nanocomposite called MMIP (magnetic molecularly imprinted polymer)/GO was created by combining EGDA as a cross-linker, GO, Fe_3O_4, and the AD medication rivastigmine with acrylamido-2-methyl-1-propanesulfonic acid (AMPS) as a functional monomer. In contrast to magnetic non-imprinted polymer (MNIP)/GO, MMIP/GO demonstrated a maximum adsorption capacity of 71.41 mg/g for rivastigmine, with a selectivity factor of 1.98 [106]. Rehmani et al. conducted a parallel investigation in which they reported the development of novel pH-sensitive porous hydrogel beads using water-soluble gum tragacanth (GT) polysaccharide and GO nanosheets as the foundational materials, with $CaCO_3$ particles serving as the solid porogen, Ba^{+2} and Ca^{+2} ions as crosslinking agents, and rivastigmine as the AD drug molecule. These hydrogel beads showed good pH sensitivity during drug release tests in simulated gastric (45% at pH 1.2) and intestinal (97% at pH 7.4) environments [107]. Additionally, Puerarin-loaded GO nanosheets exhibit exceptional drug-loading capacity, versatile surface functional groups, and high biocompatibility. Puerarin was successfully transported across the BBB and into the brain using lactoferrin as the targeted ligand, capable of attaching to the vascular endothelial receptor on the barrier. This multifunctional brain-targeted drug delivery system (Lactoferrin-GO-Puerarin) has demonstrated effectiveness and safety for treating PD, supported by both *in vivo* and *in vitro* findings [108]. FTY720 is the previously approved oral therapy drug for relapsing kinds of MS, in addition to AD and PD. PEG was used to conjugate nano GO (nGO), and the resulting PEG-nGO was functionalized with the MS medication FTY720 *via* an amide bond to create drug conjugated. The FTY720-conjugated PEG-nGO's *in vitro* drug release was assessed at 37 °C in PBS with pH values of 7.4 and 4.6 [109]. A study investigated the adsorption of MS medications (ampyra, cladribine, and cyclophosphamide) on Ni-doped graphene nanosheets using liquid phase DFT calculations. Negative adsorption energies (25.16–110.77 Kcal/mol) for all G-Ni-drug complexes suggest a favorable adsorption process. Results highlight cladribine's stronger interaction with G-Ni, suggesting graphene-nickel as a potential drug carrier for MS [110] (Fig. 3).

Role of Carbon-Based Nanomaterials in Tissue Engineering in NDDs

In tissue engineering, the rising popularity of carbon-based nanomaterials such as graphene, carbon nanotubes, and metal-organic frameworks underscores their potential for enhancing biomaterial properties and enabling precise control over cellular interactions, paving the way for advanced therapeutic solutions [111]. Carbon nanomaterials, with their unique physicochemical properties, offer promising potential for developing tissue engineering scaffolds due to their exceptional biocompatibility and versatility in mimicking biological environments, fostering tissue regeneration and repair [112]. Various nanomaterials have been extensively employed for diverse applications including mitigating neuroinflammation, regulating stem cell differentiation, and modulating synaptic plasticity. Furthermore, they play a crucial role in promoting neural cell and tissue regeneration post-injury. These applications highlight the multifaceted potential of nanomaterials in addressing various aspects of NDDs [113]. For example, liposome carrying Paclitaxel (PTX) presents a potential therapeutic approach for spinal cord injury repair utilizing neural stem cells, showing promise in promoting axon regeneration post-injury. The liposome carrying PTX enhanced neuronal differentiation *via* the Wnt/β-catenin signaling pathway fostering motor and sensory functional recovery in rat models [114]. Another investigation by Olguin et al. revealed that the inclusion of liposomes altered the characteristics of hydrogels based on CS concentration and lipid type, directly impacting their ability to support the viability and differentiation of PC12 neuronal cells. This finding suggests a novel approach for employing these hydrogels in neural tissue engineering applications [115]. Multifunctionalized CS-based polymeric micelles delivered pVGF to the brain, where VGF plays crucial roles in neurogenesis, learning, synaptic, and cognitive functions. In vivo transfection results showed effective delivery, brain cell transfection, and VGF expression

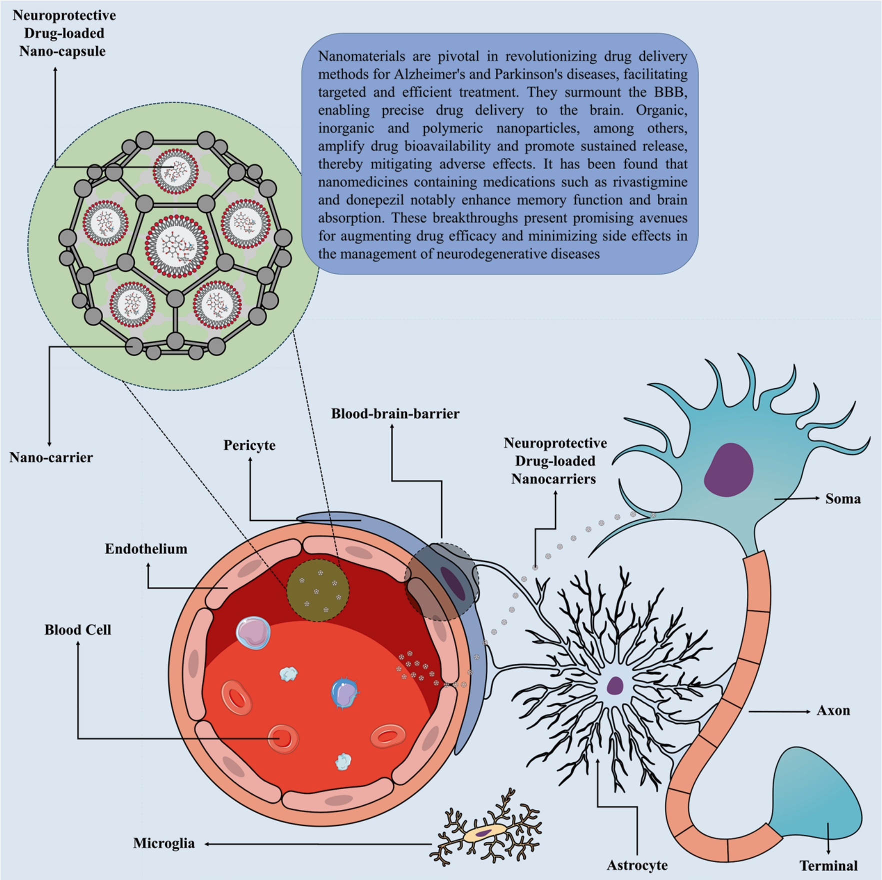
Neuroprotective Drug-loaded Nano-capsule
Nanomaterials are pivotal in revolutionizing drug delivery methods for Alzheimer's and Parkinson's diseases, facilitating targeted and efficient treatment. They surmount the BBB, enabling precise drug delivery to the brain. Organic, inorganic and polymeric nanoparticles, among others, amplify drug bioavailability and promote sustained release, thereby mitigating adverse effects. It has been found that nanomedicines containing medications such as rivastigmine and donepezil notably enhance memory function and brain absorption. These breakthroughs present promising avenues for augmenting drug efficacy and minimizing side effects in the management of neurodegenerative diseases
Nano-carrier
Pericyte
Blood-brain-barrier
Neuroprotective Drug-loaded Nanocarriers
Soma
Endothelium
Blood Cell
Axon
Microglia
Astrocyte
Terminal

Fig. 3 Role of nanomaterials in targeted and precise neuroprotective drug delivery in NDDs: Nanomaterials offer a crucial avenue for targeted and precise neuroprotective drug delivery in different NDDs. Their nano-sized dimensions enable them to traverse biological barriers like BBB, and reach specific regions of the brain with enhanced accuracy. Through surface modifications and functionalization, nanomaterials can encapsulate and deliver therapeutic agents directly to affected neuronal cells. This targeted approach minimizes systemic toxicity and maximizes drug efficacy, holding promise for improving treatment outcomes in NDDs. Harnessing nanomaterials for drug delivery represents a cutting-edge strategy in combating these complex NDDs

after noninvasive intranasal administration. Hence, the micelle-mediated approach emerges as a promising alternative for developing therapeutics in tissue engineering for AD [116]. Apart from micelles and liposomes, graphene nanomaterials like GO and rGO-based scaffolds are up-and-coming systems because of their outstanding therapeutic promise in neuronal tissue regeneration therapy. Nevertheless, a number of challenges still need to be addressed, such as immune response, cytotoxicity, biodistribution, and biotransformation [117]. Also GO enhanced the long-term potentiation from the perforant pathway to the dentate gyrus in the hippocampus, improving memory and learning deficits in AD rats. Furthermore, GO decreased p62 protein levels and increased the expression of Beclin-1 and autophagy-related LC3II/LC3I. In order to encourage autophagy, GO also suppressed the PI3K/Akt/mTOR signaling pathway. These results have shown that GO may play a part in AD neuroprotection [118]. Comparable to AD, partly rGO-films were found to stop the loss of dopaminergic (DA) neurons and the drop in α-synuclein levels in an oxidative stress-induced PD model. Notably, the rGO-film increased Tuj1 levels and the expression of transcription factors that are specific to DA neurons in the midbrain, such as Pitx3, Lmx1a, and Lmx1b [119]. According to a recent study, freshly created PCL membranes that have been enriched with rGO are suitable for use as scaffolds in simulated in vitro brain regeneration when cultured for a year at 37 °C in PBS [120]. In a separate study, 2D-GO nanosheets were applied as coatings on poly-L-lactide (PLLA) nanofibers, enhancing surface roughness to promote PC-12 neuronal adhesion and proliferation. This approach facilitates improved neuronal attachment and growth, offering potential applications in tissue engineering and neural regeneration research [121]. Magaz et al. demonstrated the potential of GO/rGO/silk-based micro/nano-fibrous scaffolds in neural tissue engineering, owing to their resemblance to the extracellular matrix (ECM) structure. This mimicry offers promising prospects for enhancing neural regeneration and tissue repair, suggesting a viable option for advancing neuroregenerative therapies [122]. Moreover, a porous gelatin structure produced by the thermally induced phase separation (TIPS) process was filled with an aerogel made of hollow gelatin oxide. Neuronal differentiation on the scaffold surface was approximately 87%, compared to 20% on the control, indicating a potential platform for brain tissue engineering [123] (Fig. 4).

4.2 Role of Polymeric Nanomaterials in Neurodegenerative Diseases (NDDs)

Polymeric nanoparticles are pivotal in managing neurological disorders by improving therapeutic delivery and efficacy, and tissue engineering, particularly. They provide targeted drug delivery, reduced toxicity, stability, and high drug loading capacity, promising enhanced interventions for NDDs such as AD, PD, MS etc. [124]. Biopolymers are increasingly employed in medicine, facilitating drug delivery by enhancing cellular uptake and preventing degradation. Overcoming the BBB remains a challenge in drug delivery for NDDs. Various strategies are being explored to bypass or traverse the BBB while minimizing post-delivery toxicity, emphasizing the importance of preserving normal physiological processes [125] (Table 1).

Role of Polymeric Nanomaterials in Biosensing in NDDs

The capacity to customize the size and structure of nanoscale materials, along with their unique capabilities, has led to the development of innovative modified transducers in recent decades. Nanomaterials are known to possess exceptional

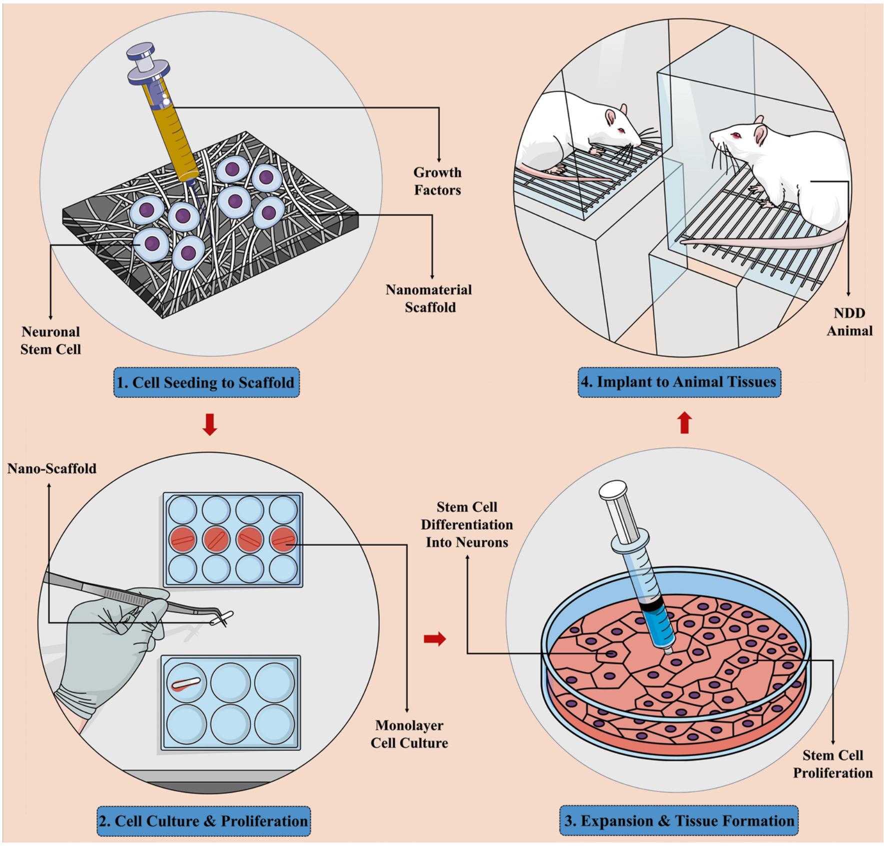
Growth Factors
Nanomaterial Scaffold
Neuronal Stem Cell
1. Cell Seeding to Scaffold
NDD Animal
4. Implant to Animal Tissues
Nano-Scaffold
Stem Cell Differentiation Into Neurons
Monolayer Cell Culture
Stem Cell Proliferation
2. Cell Culture & Proliferation
3. Expansion & Tissue Formation

Fig. 4 Role of nanomaterials in designing Scaffold and providing Niche for neuronal tissue engineering in NDDs: nanomaterials play a vital role in designing scaffolds and providing a conducive niche for neuronal tissue engineering in NDDs. Their nanostructure enables precise control over scaffold properties, such as porosity and surface topography, crucial for supporting neuronal growth and regeneration. Nanomaterial-based scaffolds mimic the extracellular matrix, promoting cell adhesion, proliferation, and differentiation in damaged neuronal tissue. By facilitating interactions between cells and their microenvironment, nanomaterial scaffolds hold promise for enhancing neural repair and regeneration in NDDs. Leveraging nanotechnology in tissue engineering presents an innovative approach towards addressing the complexities of NDDs

electrical, mechanical, thermal, and optical properties due to quantum-size effects. As such, they are regarded as a highly appealing means of advancing the development of biosensors with improved analytical capabilities [14]. Polymers, with their enduring stability and versatile functionalization capabilities, are widely harnessed in developing biosensing platforms, leveraging both synthetic and natural variants for diverse applications in biotechnology and healthcare [126]. For example, in a study, it was discovered that coassembled CS and hyaluronic acid nanoparticles cross-linked with glutaraldehyde functioned as a theranostic agent for both *in vitro* and *in vivo* imaging/probing and Aβ fibrillization suppression. Recently, due to its many unique properties, such as its high biocompatibility, excellent electrochemical redox behavior even in neutral solutions, good solubility in organic solvents, and environmental stability has been investigated for sensor applications [127]. In another study, highly magnetic zinc-doped magnetite nanoparticles were encapsulated within a PLGA matrix to produce novel magnetic beads with superior magnetization. These magnetic beads were meticulously functionalized with affinity proteins, showing enhanced antibody capture capability for Tau protein [68]. Additionally, a U-shaped fiber optic probe, immobilized with AuNP and functionalized with a CS film, served as a sensing platform for α-synuclein detection. The chitosan film, acting as a sensitive and selective polysaccharide bioreceptor, enabled detection of monomers and fibrils independently up to 70 nM. It could also differentiate between them in mixtures with fibril to monomer ratios ranging from 0.1 to 10, achieving results within 15 minutes [128]. Similarly, Beccarin et al. developed a sensor using a glassy carbon electrode modified with rGO, carbon black, and a CS film. This electrode facilitated determination of dopamine using square-wave voltammetry, with showing an anodic peak at 0.25 V. Analytical curves were linear in the range from 3.2×10^{-6} to 3.2×10^{-5} mol L^{-1} for dopamine with detection limits of 2.0×10^{-7} mol L^{-1} [129]. In a separate study, Kalinke et al. reported about the enhanced performance of PLA-graphene electrodes (PLA-G), including increased surface area, electron transfer rate, and edge site quantity for dopamine. The electrode exhibited a broad linear range and low detection limits for dopamine in synthetic urine and human serum [130]. Zhang et al. reported on the construction of a polyaniline (PANI)/Au nanocomposite modified nanoelectrode for sensitive dopamine nanosensor design. When ascorbic acid and dopamine were oxidized in a neutral pH solution, the modified nanoelectrode demonstrated outstanding electrocatalytic activity [131]. A sensitive electrochemical sensor was developed using a poly(o-methoxyaniline)-gold (POMA-Au) nanocomposite for simultaneous detection of dopamine. The sensor demonstrated enhanced electrochemical behavior for the oxidation of dopamine, with reduced over-potentials and clear separation of their overlapping peaks. POMA-Au/GCE sensor showed wide linear ranges and low detection limits for dopamine (10.0 to 300.0 μM & 0.062 μM) [132] (Fig. 2).

Role of Polymeric Nanomaterials in Drug Delivery in NDDs

Polymeric nanoparticles offer controlled and targeted drug delivery, synthesized from biodegradable polymers for enhanced bioavailability and protection against environmental factors. Tailored release mechanisms enable precise targeting of tissues or cells, maximizing therapeutic efficacy in treating diseases such as NDDs and improving nutraceutical delivery [66]. Bhattamisra et al. developed rotigotine-loaded CS nanoparticles (RNPs) for nose-to-brain delivery, addressing challenges of limited water solubility and low bioavailability in PD treatment. Through cell-based studies, they demonstrated RNPs' enhanced neuronal absorption, antioxidant, and neuroprotective properties, suggesting a promising therapeutic approach for PD [133]. Curcumin's potential in AD treatment is hindered by low solubility and bioavailability. To address this, Dhas et al. developed CS-functionalized PLGA core/shell nanoparticles (CH@Cur-PLGA C/S NPs) for intranasal delivery of curcumin, showing enhanced efficacy, cellular uptake, and ROS scavenging activity, promising for AD therapy [134]. To boost Astragaloside IV's (ASI) efficacy in MS, β-asarone modified ASI-loaded CS nanoparticles (ASI-βCS-NP) were tailored for nose-to-brain delivery. These nanoparticles, sized at 120 nm with ζ potential ranging from +19 to +25 mV, exhibited efficient nose-to-brain targeting. Intranasally administered ASI-βCS-NP significantly improved symptoms, reduced inflammation, and promoted remyelination in a mice EAE model, suggesting potent efficacy against MS [135]. Memantine, approved for moderate to severe AD, has not been fully effective. To address this, PLGA nanoparticles were considered as a potential solution to enhance drug action at the target site and reduce adverse effects. The development resulted in memantine crossing the BBB when loaded into biodegradable PLGA nanoparticles *via* the double emulsion method and surface-coated with polyethylene glycol, with both *in vitro* and *in vivo* data demonstrating sustained drug delivery to the target tissue [136]. In another study, CS nanoparticles incorporating rivastigmine, an AD drug prepared *via* spontaneous emulsification, exhibited a mean particle size of 47 ± 4 nm with a positive ζ potential. Coating with polysorbate 80 slightly reduced surface charge, while a biphasic release pattern was observed for drug release. Biodistribution studies indicated that coating with 1% polysorbate 80 altered nanoparticle uptake by various organs [137]. AD drives the use of 7-Methoxytacrine (7-MEOTA) as an approved treatment. Investigation of polycaprolactone nanocapsules (7-MEOTA@PCL) unveiled a 65% drug loading, 237 nm average particle size, negative surface charge, stable morphology, and controlled release kinetics following Fick's law [138]. In another study, combining formulation factors enhanced galantamine (GAL) bioavailability *via* intranasal delivery using nanosized polymeric particles with polyacrylic acid (PAA) and taurodeoxycholate (TDC). The optimized formulation, with equal ratios of PAA and TDC and 20 mg GAL, exhibited a particle size of 185.55 ± 4.3 nm, complete drug release within 2 hours, and improved brain cell uptake in AD [139]. Polyacrylic acid conjugated cerium oxide (PAA-CeO) nanoparticles, synthesized at 50–60 nm with a ζ potential of −35 mV, show neuroprotective potential against H_2O_2 and 6-OHDA-induced PD neuronal damage, inhibiting necrotic processes and modulating caspase-3 activity in the 6-OHDA model, without direct ROS inhibition in the H_2O_2 model, suggesting a multifaceted mechanism of action [140]. Moreover, Yoncheva et al. investigated poly(methyl methacrylate-co-acrylic acid) (PMMA-AA) copolymers for forming hollow particles and as a curcumin delivery system in PD rats. Optical micrographs revealed spherical hollow microparticles (1–7 μm), and encapsulated curcumin exhibited enhanced protective effects in rat brain synaptosomes, including increased synaptosomal viability and intracellular glutathione levels [141] (Fig. 3).

Role of Polymeric Nanomaterials in Tissue Engineering in NDDs

Polymeric nanomaterials play a pivotal role in tissue engineering by serving as scaffolds that promote tissue growth and deliver bioactive factors. Their versatility and suitability for regeneration across various tissues make them increasingly popular choices in tissue engineering endeavors [142]. Conductivity in these nanomaterials can be controlled, as evidenced by the fabrication of electrospun scaffolds using PANI, PCL, and gelatin, yielding fibers with diameters between 112 and 189 nm. Electrical stimulation on these scaffolds has shown to enhance neural stem cell proliferation and facilitate neurite outgrowth, offering potential for advanced tissue engineering applications [143]. In a study, Ranjan et al. reported that PLGA microfiber scaffolds with FAD iPSC-derived neurons facilitate AD simulation *in vitro*, boasting large pore sizes for deep cellular infiltration and homogeneous distribution. In 3D cultures, stem-cell derived neural progenitor cells showed accelerated neuronal development, increased survival, and decreased proliferation compared to 2D monolayers. Furthermore, 3D scaffold cultures exhibited higher levels of pathogenic $A\beta_{42}$ and p-tau, suggesting robust recapitulation and accelerated early-stage AD pathogenesis [144]. Unconjugated PLGA nanoparticles exhibit potential therapeutic benefits in both cellular and animal models of AD by targeting multiple components of the Aβ axis. Findings indicate that under non-physiological temperatures, these nanoparticles can either promote or inhibit the breakdown of Aβ aggregates. Additionally, PLGA demonstrates a unique ability to shield neurons from Aβ-induced toxicity, underscoring its promise as a therapeutic intervention against AD pathogenesis [145]. In a similar study, it was found that after acute injection, native PLGA accumulates in Aβ-labelled neuritic plaques, mirroring *in vitro* findings. In 5xFAD mice, 28-day native PLGA treatment reduces Aβ plaque load and enhances cognitive function, with no notable changes in APP or Aβ levels in the cerebellum of 5xFAD mice or other brain regions of control mice [146]. The rapid synthesis of novel CS-gelatin-green tea extract (CS-Gel-GTE) composite particles *via* angle-dependent electrospray technique demonstrates outstanding antioxidant properties for PD therapy. These particles enhance cell survival, reduce apoptosis, inhibit ROS production, elevate TH enzyme expression, and lower α-synuclein protein levels [147]. Crosslinked nanofibers of CS and gelatin, mimicking the natural ECM, were developed for neural tissue engineering, exhibiting adequate biocompatibility confirmed *via* SEM, contact angle, porosity, and PC-12 cell assays. Moreover, seeding OLN-93 cells on these nanofibers with autoreactive T-cells replicated oligodendrocyte reduction akin to MS pathology, validated by increased expressions of CD80, CD86, and IL-12p40 [148]. It has been found that PLA/CS scaffolds support neural cell development, inducing PC12 cells into neural-like cells (NLCs) with NGF, bFGF, and conditioned medium. PC12 cells successfully adhere, grow, and differentiate on these scaffolds. Transplantation of PC12-derived NLCs into mouse brain lateral ventricles reduces clinical signs of EAE, evidenced by decreased demyelination, axonal damage, and inflammation on histological analysis [149]. Bone marrow-derived mesenchymal stem cells genetically modified to overexpress neurotrophin-3 (NT-3) differentiate into neurons on PLGA microcarriers with collagen. Increased TH production confirms successful NT-3 overexpression, promoting dopamine neuron development. Efficient adhesion and dopamine detection validate effective differentiation, presenting a promising PD treatment [150]. Fabricated electrospun nanofibers of PCL, CS, and PPy blend exhibited improved surface characteristics and bioactivity. Chitosan incorporation notably enhanced scaffold hydrophilicity by reducing contact angle up to 66%. The *in vitro* assays revealed enhanced cell attachment, spreading, proliferation (up to 356% compared to pure PCL), and neurite extension on the PCL/CS/PPy nanofibrous scaffold [151] (Fig. 4).

4.3 Role of Inorganic Nanomaterials in Neurodegenerative Diseases (NDDs)

Inorganic nanomaterials possess exceptional physicochemical properties, such as catalytic, optical, thermal, electrical, or magnetic characteristics, making them promising for various biomedical applications like drug delivery, diagnostics, imaging, and therapy. However, their long-term accumulation in healthy tissues can lead to toxicity, hindering widespread clinical use [152]. In recent years, the development of biodegradable and clearable inorganic nanomaterials has emerged as a solution to mitigate such risks. Understanding the design of these materials and their metabolic pathways within the body is crucial for expanding theranostic applications and advancing clinical trials. Therefore, the development of biodegradable and clearable inorganic nanomaterials is imperative to unlock their full potential in biomedicine [153] (Table 1).

Role of Inorganic Nanomaterials in Biosensing in NDDs

Inorganic nanoparticles, devoid of carbon, offer distinct advantages in the field of biosensors. They are hydrophilic, non-toxic, and compatible with living systems. Moreover, their stability surpasses that of their organic counterparts. Common examples of inorganic nanomaterials that are used in biosensors encompass iron, silver, gold, and titanium nanoparticles, among others [154]. For example, AuNPs possess unique properties that drive growing interest in their application. Their optical properties enable the construction of diverse optical biosensors. Additionally, their biocompatibility, conductivity, catalytic properties, high surface-to-volume ratio, and density facilitate the development of electrochemical and piezoelectric biosensors, enhancing analytical performance compared to other designs [155]. For example, an interdigitated microelectrodes as an impedance biosensor for blood-based Aβ detection was developed using AuNPs sandwich assay. This approach demonstrated logarithmically linear sensitivity, enhancing detection limits approximately 2.87-fold with a success rate of 74.84%, demonstrating high sensitivity and selectivity for Aβ detection in plasma samples of AD [156]. In a separate study, an

AuNP-polyglutamic acid-modified indium tin oxide (ITO)-based disposable neurobiosensor system was developed for the detection of CSF α-synuclein, a crucial biomarker for PD, exhibiting highly linear and sensitive charge transfer resistance (R_{ct}) changes within the concentration range of 4–2000 pg mL^{-1}, with a limit of detection of 0.135 pg mL^{-1} [157]. Another investigation introduced a novel approach for detecting dopamine using a AgNP modified electrode coated with graphene oxide. The process involved sequentially modifying the surface of an (ITO) electrode with AgNPs and GO through electrochemical deposition. This newly developed biosensor exhibited enhanced electrochemical signal amplification, particularly at low dopamine concentrations, outperforming prior biosensors [158]. In addition to gold nanoparticles, a novel and thermostable biosensor utilizing biomimetic mineralization HRP@Fe-MOF as a signal probe offers superior stability, sensitivity, specificity, and long-term activity at 37 °C compared to existing assays for measuring Aβ oligomers. The Fe(II)-based MOF-armored HRP addresses thermal instability, enabling successful Aβ oligomer measurement in blood serum samples, promising improved detection for early AD diagnosis and treatment decisions [159]. In another study, a novel method was employed to fabricate α-Fe2O3@erGO nanocomposite, and a modified sensor was created through direct electrochemical reduction. This sensor exhibited high sensitivity and selectivity for dopamine detection. Notably, the modified sensor achieved a low detection limit of 24.70 nM for dopamine, which can be potentially used for the early diagnosis of PD [160]. Apart from iron oxide nanoparticles, a simple, cost-effective nanostructuring technique was proposed to construct a photoelectrochemical (PEC) sensor *via* solution-based hydrothermal growth of titanium dioxide (TiO_2) nanobrush (TiO_2-NB). The TiO_2-NB PEC sensor demonstrated high sensitivity (114.8 μA/(ng mL^{-1})) and low LoD (26.3 ng mL^{-1}) for detecting a wide range of $A\beta_{1\text{-}28}$ peptides. This offers a label-free, rapid, sensitive, and noninvasive method overcoming conventional techniques' limitations in AD diagnosis [161]. A highly sensitive sensor utilizing electrospun TiO_2 nanofiber/graphite oxide paste/GCE was developed for voltammetric determination of levodopa (L-DOPA). The anodic peak currents measured by differential pulse voltammetry (DPV) showed a good linear relationship in the concentration range of 3×10^{-7} to 6×10^{-5} M of L-DOPA, with a LOD of 15.94 nM and sensitivity of 0.0806 μA μM^{-1}. This method was effectively applied to quantify L-DOPA in human CSF, blood serum, and plasma [162]. A highly sensitive impedimetric immunosensor for detecting Anti-Myelin Basic Protein (Anti-MBP), an autoantibody associated with MS, was developed. This novel sensor immobilizes MBP on Gelatin and Gelatin-TiO_2 modified platinum electrodes. The designed immunosensor exhibits wide linear detection ranges (0.975–2500 ng mL^{-1} for gelatin-MBP and 0.4875–2500 ng mL^{-1} for gelatin-TiO_2-MBP electrodes) and low detection limits, offering promising applications in MS diagnosis [163] (Fig. 2).

Role of Inorganic Nanomaterials in Drug Delivery in NDDs

Inorganic nanomaterials offer ideal drug delivery platforms, ensuring efficient and safe therapy with minimal systemic side effects. Their unique physicochemical properties, including easy preparation, storage stability, and biocompatibility, make them suitable for delivering small-molecule drugs, nucleic acids, and proteins. Numerous inorganic nanostructure-based drug delivery platforms have been developed [164]. For example, evidence suggests curcumin can protect cells from Aβ-mediated neurotoxicity by inhibiting Aβ aggregation, but its low stability and water-solubility hinder targeted uptake and bioavailability. To address this, a novel curcumin delivery system employing Fe_3O_4@carbon dots nanocomposite (Fe_3O_4@ CDs) as the carrier has been developed for Alzheimer's therapy. curcumin- Fe_3O_4@ CDs exhibit a strong affinity toward Aβ, effectively inhibit Aβ fibrillation, reduce ROS production, mitigate neurotoxicity in PC12 cells, and restore nerve damage while maintaining neuronal morphology [182]. Another study investigated a multifunctional conjugate utilizing magnetite iron oxide nanoparticles (MIONPs) for cellular delivery of siRNA targeting BACE1 gene expression implicated in AD. SiRNA immobilized on PEGylated MIONPs, with co-immobilized translocating protein OmpA, showed significant BACE1 suppression in HFF-1 cells without cytotoxicity, likely mediated through endocytosis pathways with enhanced endosomal escape via proton-sponge effect or direct translocation by OmpA/PEGylated nanoparticles [165]. Qing et al. developed a superparamagnetic iron oxide nanocube (SPIONs)-based delivery system to effectively deliver epigallocatechin gallate (EGCG) for inhibiting α-synuclein aggregation in PD treatment. This approach overcomes EGCG delivery challenges, including limited accumulation in dopaminergic neurons and high binding affinities with membrane proteins. The system significantly inhibited α-synuclein aggregation and increased dopaminergic neuron count in PD [9]. In a separate investigation, Guan et al. encapsulated magnetic Fe_3O_4 nanoparticles with N-isopropylacrylamide derivative (NIPAm-AA) along with short hairpin RNA (shRNA), and attached NGF onto the nanoparticle surface *via* electrostatic interaction. These nanoparticles, carrying shRNA targeting α-synuclein, demonstrated effective repair in an MPTP-induced PD model through NGF receptor-mediated endocytosis [166]. In a separate study, Hu et al. engineered Fe_3O_4 nanorods for magnetically guided delivery of tissue plasminogen activator (tPA) in early acute ischemic stroke intervention. tPA was covalently conjugated to Fe_3O_4 *via* glutaraldehyde-mediated attachment to -NH2 groups on the surface. tPA-loaded magnetic rods (tPA-MR) were administered to a distal middle cerebral artery occlusion mouse model through intra-arterial injection following $FeCl_3$-induced vessel occlusion [167]. Apart from iron nanoparticles, HEMA/PEG-DA-based hydrogels with low TiO_2 nanoparticle concentrations were synthesized through photopolymerization using different initiators. FTIR verified hydrogel functional groups, and digital microscopy along with SEM characterized their structure. Findings highlighted substantial effects of hydrogel content, initiators, and pH on swelling and release of Donepezil hydrochloride, an AD treatment drug [168]. Additionally, laponite nanocomposites enhanced brain delivery of donepezil through encapsulation, leveraging their nano-size and positive charge at a certain pH. Laponite-donepezil formulations inhibited acetylcholinesterase by approximately 82%, surpassing plain donepezil (30%), with Swiss albino mice showing increased brain uptake *via* intravenous administration [169] (Fig. 3).

Role of Inorganic Nanomaterials in Tissue Engineering in NDDs

In recent years, tissue engineering has flourished due to increased demand for replacement tissues amidst donor shortages. Researchers are crafting scaffolds to mimic extracellular matrices, vital for neuronal tissue regeneration. Inorganic nanomaterials, with varied physicochemical traits and bio-inductive potential, are gaining momentum in tissue engineering, though challenges persist in optimizing their efficacy [170]. For instance, AuNP-decorated electrospun nanofiber scaffolds have been shown to promote extended neurite outgrowth, as evidenced by the increased total length of branching trees and neurite length and distance. Additionally, neurons cultured on these nanocomposite scaffolds exhibit reduced neurites emanating from the soma and fewer branches. These findings suggest neurons prefer axonal elongation over complex branching when grown on AuNP scaffolds [170]. In another study, it was found that micro/nano-channeled PCL/PLGA film scaffolds, crafted *via* electron beam lithography, were adorned with AuNP and IKVAV-pentapeptide to enhance conductivity and hydrophilicity. Electric stimulation from a bioreactor system facilitated neural stem cell differentiation and alignment. This innovative scaffold design holds promise for fostering long-distance axonal regeneration post-neural injuries [171]. AuNPs enhanced survival of hNSCs treated with Aβ by decreasing caspase 3 and 9 activities. Additionally, AuNPs reversed Aβ-induced downregulation of neuroprotective (CREB and Bcl-2) and mitochondrial (PGC1α, NRF-1, and TFAM) gene expressions in treated hNSCs. Co-treatment with AuNPs rescued hNSCs from Aβ-mediated mitochondrial dysfunction and morphology, normalizing mitochondrial marker immunostaining and mass in differentiated hNSCs with Aβ. These effects suggest that AuNPs protect against Aβ-induced cellular impairment and mitochondrial dysfunction in hNSCs [172]. In a study, transmembrane peptide-chondroitin sulfate gold nanoparticle (TAT-CS@Au) was discovered to possess excellent transmembrane ability, inhibit $A\beta_{1-40}$ accumulation, antagonize oxidative stress, reduce aberrant tau phosphorylation, and suppress the expression of inflammatory factors. TAT-CS@Au, identified as a multi-target anti-AD drug with good cell permeability, offers new insights into the design and research of anti-AD therapeutics [173]. Gao et al. suggested investigating the biological interaction between TiO_2 films and AD biomarkers. These nanofilms were characterized both before and after exposure to $A\beta_{1-42}$. Subsequently, the study revealed that TiO_2's photocatalytic activity led to the degradation of $A\beta_{1-42}$. This process mitigated the formation of fibrils, presumably triggered by a photocatalytic mechanism, by diminishing the core structure of $A\beta_{1-42}$ in AD [174]. In a separate investigation, combining mesenchymal stem cells with TiO_2-nanowires alongside cerebrolysin (CBL), a compound containing active peptide fragments and neurotrophic factors, notably decreased Aβ deposits. This approach exhibited enhanced neuroprotective effects in AD [175]. Furthermore, a simple co-precipitation method was employed to synthesize ZnO nanoparticles, subsequently analyzed using UV-Vis spectroscopy and FTIR. Upon introduction *in vitro*, ZnO effectively rescued SH-SY5Y cells from damage induced by 6-OHDA, a PD-inducing neurotoxin by halting apoptotic processes [176]. Biocompatible PEDOT/MnO_2 nanoellipsoids enhance neurite outgrowth and enable real-time monitoring of PC12 neuronal cells. The dose of PEDOT/MnO_2 nanoellipsoids facilitates neurite outgrowth, with MnO_2 domains playing a crucial role in cell differentiation. These findings suggest potential applications of PEDOT/MnO_2 nanoellipsoids in tissue engineering for mammalian nervous systems [177]. Recent research reveals that dimanganese trioxide (Mn_2O_3) nanoparticles enhance PD treatment. Mn_2O_3 exhibits enhanced inhibition of α-synuclein fibrillization and acts as ROS scavengers following cellular uptake via the caveolin-mediated pathway. The *in vivo* studies demonstrate an 81% reduction in α-synuclein concentration in PD mice's CSF and a 2.3-fold increase in dopamine levels in their brains [178] (Fig. 4).

5 Future Perspective

The utilization of nanomaterials in addressing NDDs is encumbered by several challenges. Firstly, ensuring the biocompatibility and safety of nanomaterials is paramount due to their potential for unintended toxic effects on neurons and other CNS cells, necessitating extensive safety testing to mitigate long-term risks [179]. Secondly, overcoming the BBB to deliver therapeutic agents poses a significant obstacle, requiring the development of strategies for effective penetration without compromising BBB integrity. Additionally, achieving specific targeting of affected neurons while minimizing off-target effects remains challenging, demanding the engineering of nanomaterials tailored for precise delivery to diseased cells or brain regions [13]. Furthermore, addressing issues of clearance and biodegradation is essential to prevent the accumulation of nanomaterials in the body and brain, mitigating potential toxicity over time. Ethical and regulatory considerations surrounding informed consent, patient safety, and unforeseen consequences must also be addressed to ensure the responsible development and use of nanomaterial-based therapies [180]. Looking forward, nanomaterials hold promise for personalized and precision medicine approaches, early diagnosis and imaging, targeted drug delivery, neuroregeneration, and repair, as well as integration into brain-machine interfaces for therapeutic applications in NDDs, such as AD, PD, and MS [14].

6 Conclusion

Nanomaterials are instrumental in addressing the theranostic applications of NDDs, such as AD, PD, MS, and HD. Their distinctive characteristics facilitate precise drug delivery to the brain, effectively traversing the BBB. Engineered nanoparticles can encase therapeutic medications, safeguarding them from deterioration and enhancing their availability within the body. Furthermore, nanomaterials support early disease identification through cutting-edge imaging methods, allowing for accurate diagnosis and monitoring of disease advancement. They also present exciting prospects for neural rejuvenation and restoration by furnishing frameworks for cell proliferation and encouraging the regrowth of neurons. Additionally, nanotechnology empowers the creation of biosensors for detecting disease indicators, aiding in prompt diagnosis and tailored treatment strategies. Moreover, drug delivery systems based on nanomaterials diminish unintended effects, lessening toxicity and enhancing patient adherence. Nanoparticles can also regulate neuroinflammatory reactions, showing promise in managing the inflammatory aspect of neurodegenerative disorders. Furthermore, nanotechnology boosts the effectiveness of gene therapy by facilitating targeted delivery of therapeutic genes to affected brain regions. In conclusion, nanomaterials offer substantial potential in transforming the diagnosis, therapy, and control of NDDs, promising improved patient outcomes and quality of life.

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Balancing Innovation and Responsibility: Ethical, Legal and Social Considerations in Nanomedicine

Anjali A. Kulkarni and Swapnil C. Kamble

1 Introduction

Nanomedicine includes the use of nanomaterials in medical intervention for diagnosis, monitoring, control, prevention, and treatment of a disease according to the European Commission (EC) [38]. Yet, there is no consensus on the definition of nanomedicine. In pharmaceutical sciences, nanomedicine refers to the use of nanotechnologies in producing active pharmaceutical ingredients (APIs) as nanoscale particles, or by combining APIs with suitable nanomaterials to produce nanoscale particles that can be further formulated into versatile dosage forms. Food and Drug Administration (FDA), USA has no separate definition of Nanomedicine. Due to its close interaction with human health, Nanomedicine poses unique challenges and urgently requires a specific governance framework. The governance is further complicated by the multi and inter-disciplinary nature of Nanomedicine that impinges upon aspects being governed by different legislations. Biosafety, nanotoxicity and accessibility need to be addressed appropriately. Figure 1 tries to capture a few of the multiple dimensions of Nanomedicine (Modified from [10, 15]).

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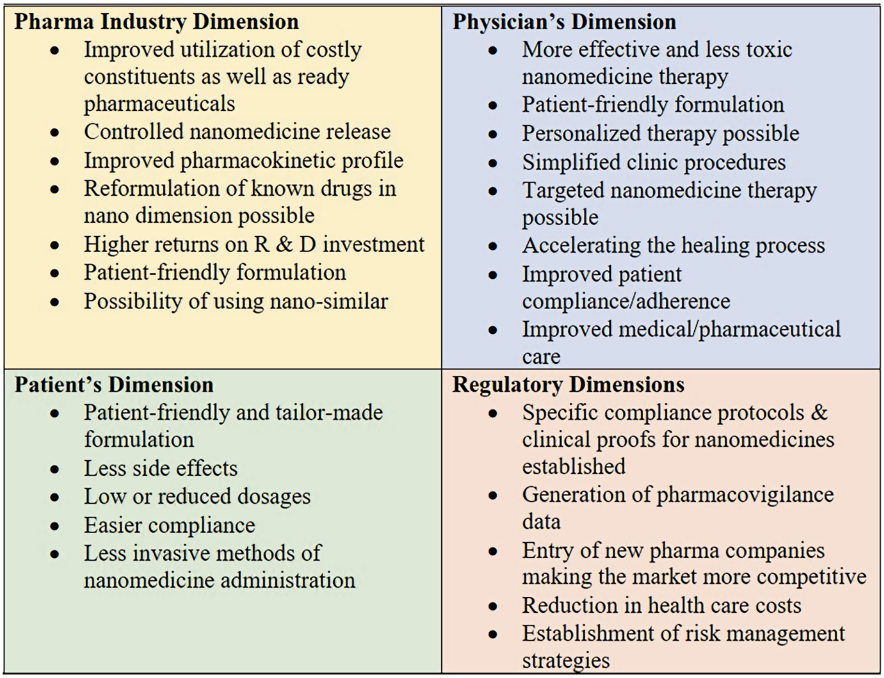

Pharma Industry Dimension
- Improved utilization of costly constituents as well as ready pharmaceuticals
- Controlled nanomedicine release
- Improved pharmacokinetic profile
- Reformulation of known drugs in nano dimension possible
- Higher returns on R & D investment
- Patient-friendly formulation
- Possibility of using nano-similar

Physician's Dimension
- More effective and less toxic nanomedicine therapy
- Patient-friendly formulation
- Personalized therapy possible
- Simplified clinic procedures
- Targeted nanomedicine therapy possible
- Accelerating the healing process
- Improved patient compliance/adherence
- Improved medical/pharmaceutical care

Patient's Dimension
- Patient-friendly and tailor-made formulation
- Less side effects
- Low or reduced dosages
- Easier compliance
- Less invasive methods of nanomedicine administration

Regulatory Dimensions
- Specific compliance protocols & clinical proofs for nanomedicines established
- Generation of pharmacovigilance data
- Entry of new pharma companies making the market more competitive
- Reduction in health care costs
- Establishment of risk management strategies

Fig. 1 Multiple Dimensions of Nanotechnology and Nanomedicine

1.1 *Importance of Ethical, Legal, and Social Issues (ELSI) of Nanomedicine*

Ethics (E) include the societal norms which may change over the long term but are stable in the short term. Generally E forms the basis of Law (L). L is quite adaptable and changeable, based on feedback received from the society (S). When emerging technologies such as Nanomedicine are introduced in the S, current L may not be able to cover them. So, a new code of Ethics needs to evolve with respect to the emerging technologies and their amalgamations, which again will cyclically lead to new legislations, societal awareness, creation of enterprise and changes in the life-style (https://elsi.osaka-u.ac.jp/en/what-is-elsi).

Many countries like USA (https://www.nano.gov/sites/default/files/pub_resource/riskmanagementandelsi.pdf), countries of EU, Japan etc. have established separate committees to address ELSI but in India, we could not find any reference for such a committee. Reports from these groups highlight the importance of increasing the societal awareness of Nanomedicine through special campaigns in social and print media as well as involvement of respectable NGOs and social science researchers in the research projects right from the beginning. These groups suggested that scientists should collect societal perception and awareness data from people of different financial, educational and geographical backgrounds. People from both developed as well as developing nations should be included in the sample. Addition of ELSI principles and case studies in educational syllabi of Nanotechnology and Nanomedicine course would be a great step. All of these steps would help in the ultimate goal of a global ELSI framework.

2 Ethical Considerations in Nanotechnology

2.1 Ethical Challenges in Nanotechnology and Nanomedicine Research

Despite growing interest in the ethics of nanotechnology since the early 2000s, few recent studies have comprehensively reviewed ethical and legal concerns related to the research and development of nanomedical technology. Table 1 compiles pertinent risks and perceptions related to nanomedicines [15].

Table 1 Risks and Perceptions in Nanomedicine

Risks	Perceptions
Harm of exposure & health implications for nanoscale medicines	The nanomedicines can undergo enzymatic degradation, Can be uptaken by scavenging phagocytic cells, May lead to abnormal blood flow and hydrostatic pressure at the target sites, May activate molecular and ionic efflux pumps that expel drugs from target cells.
Transparency in sharing nano-medicine related data with patients	Safety and risk concerns
Patient consent	Do the patients give their consent after detailed information about treatment? Do these details include all ‘pros’ and ‘cons’?
Patient privacy	Is patient privacy of utmost importance?
Nanomedicine access equity	Do the patients from all strata of the society receive the same detailed information
Classification of nano-medical products	Absence of clear consensus in primary definitions of nanomedicines
Precautionary principle	Are GMP practices being followed? Is there clarity regarding ‘uncertain’, ‘unknown’ and ‘unpredictable’ aspects of nanomedicine?
Issue of nanosimilars	Have the nanosimilars been stringently evaluated?

The Dual-Use Dilemma

As per WHO (2021), Dual-Use Research of Concern (DURC) is defined as any life science research that is intended for benefit of humankind, but which might be misapplied to cause harm across multiple domains. Nanotechnology and Nanomedicine perfectly fall under this realm. The same wonderful technology that can design life-saving drugs, and targeted drug delivery systems, can unfortunately create deadly pharmaceuticals with neurotoxic effects, with risks of both accidental and deliberate harm. Further, nanomaterials can accumulate and cause ecological and environmental harm. Unseen, unmanned aerial and aerosolized nano-bioweapons are an actual danger. Nanotech's dual use makes things complicated for all the stakeholders. On the one hand, there is a huge opportunity for positive change, but on the other hand, there are very real dangers associated with it. Also, nanotechnology is developing faster than the policies that can regulate it. In 2020, WHO had issued guidance on biosafety and biosecurity in biomedical laboratories, and it envisaged that the 'safety-by-design' approach was the only way forward for nanotechnological and nanomedical research, wherein risks and implications are considered right at the outset of a research project and they are made an integral part of the study design rather than an afterthought.

Environmental and Health Concerns

Nanopollution (waste generated by nanodevices or during nanomaterials manufacturing process) can be very dangerous to environment because of its miniscule size. It can float in the air and might easily penetrate animal and plant cells as a nano-aerosol, thereby causing unknown effects. Most human-made nanoparticles do not have an equivalent entity existing in nature, preventing living organisms from processing and detoxifying them. To properly assess the health hazards of engineered nanoparticles, the whole life cycle of these particles needs to be evaluated, including their fabrication, storage and distribution, application and potential abuse, and disposal. The impact on humans or the environment may vary at different stages of the life cycle. Environmental assessment is justified as nanoparticles present novel environmental impacts.

2.2 Responsible Conduct in Nanotechnology and Nanomedicine Research

Public accountability, public participation and transparency are the cornerstones of ethical dimensions. NGOs dealing with environment, public policy, health, various policy groups, news and social media platforms are involved. Studies on Ethics form a less highlighted section in Science and Engineering curricula and emphasis on regular courses about current scenarios in Ethics is a must for in-service researchers. Legal contexts (consumer legislation, occupational health legislation, criminal negligence claims against corporations, laws regarding new technology development-production and dissemination) need to be addressed. Just like the World Commission on the Ethics of Scientific Knowledge and Technology (COMEST) (https://www.unesco.org/en/ethics-science-technology/comest), national level committees on Ethics in Nanotechnology and Nanomedicine must be set up by India. They should develop special public outreach programs in multiple Indian languages, for people of different educational backgrounds.

3 Legal Frameworks in Nanotechnology

3.1 Regulatory Landscape

Regulating the field of nanoparticles and nanomedicine is a difficult task due to the immense structural and functional diversity of nanomaterials, such as nanoparticles, nanocrystals, nano-complexes, nano-emulsions, polymers, liposomes, dendrimers, metal colloids, micelles, quantum dots, fullerenes, carbon nanotubes, and virosomes; to name just a few. All of them need to have 'biocompatible' surfaces so as not to trigger an undesired response in the organism when introduced in its body. When such diverse nanomaterials are either themselves used as therapeutics or combined with drugs to act as nanocarriers for the drugs, new modalities come into picture. E.g. usual drug formulations (tablets, capsules, syrups) become effective only after their successful transport across biological membranes. But nanoparticles whether used as themselves or as carriers, become effective right at the cell membrane boundary. Their pharmacodynamic and pharmacokinetic properties are completely different due to their small and varied sizes, huge surface areas, enhanced reactivity and catalytic activity, faster electron/ion transport, altered thermal, optical, and magnetic properties, varied density and dispersion properties, surface decorations with drugs and/or haptens, enhanced biological or chemical activity; novel quantum mechanical properties. Due to the very same advantageous properties, nanoparticles and nanomedicines may raise concerns for high toxicity profiles owing to their unknown, unpredictable, or unwanted distribution in tissues and interactions with biological macromolecules and cellular constituents. For example, nanoparticles of 10 nm size were preferentially found in blood, liver, spleen, kidney, testis, thymus, heart, lung, and brain, while larger particles were detected only in spleen, liver, and blood [21, 27].

Effects due to nanoparticles further change with respect to change in their sizes and surface characteristics, impacting the filtering capacity of phagocytes, the transport effects of capillary structures, or adhesion to proteins or other molecules in biological fluids. For example, small nanoparticles (below 20–30 nm size) are rapidly cleared by renal excretion, while 200 nm or larger particles are more efficiently taken up by the mononuclear phagocytic system (reticulo-endothelial system) located in the liver, spleen, and bone marrow [1, 26]. Detailed studies are required to investigate penetration of nanomaterials in cells and tissues, and their subsequent distribution, degradation, and excretion. A new field in toxicology termed 'Nanotoxicology' has emerged, which aims at studying the above-mentioned effects and interactions of nanomaterials with biological systems. In some cases, these interactions can be predicted; while in others, they are unexpected. The latest trend is to use AI-ML tools to determine these multiple variables [37]. Nanomedicines' development, manufacturing, and marketing fall under the provisions of the existing regulatory frameworks on medicinal products in both the European Union (EU) and the United States (US) [21, 27].

International Regulations

Because of the all-encompassing aspect of Nanotechnology, UNESCO took an initiative to form a regulatory group of member nations called the World Commission on the Ethics of Scientific Knowledge and Technology (COMEST) in 2003. After multiple meetings of philosophers, scientists, and policy makers, the first draft of Policy Recommendations on Nanotechnologies and Ethics was presented [43]. Ethical concerns of nanotechnology are high because of the following reasons:

- The nanoparticles are invisible to the naked eye, making them difficult to trace and control in the absence of sophisticated technology.
- Their rate of development is rapid and long-term studies about their impact on humankind and the environment are lacking. The impact is envisaged to be global and not restricted to the countries using nanotechnology.
- Military and security uses of nanotechnology may raise global concerns.
- Inequalities between developing and developed countries regarding usage of nanotechnologies is feared, leading to 'nano-divide'.

Besides the common global guidelines, different countries and regions have drafted their own regulatory guidelines with respect to nanotechnology and nanomedicine. For example, specific European guidelines and regulations; OECD guidelines for cosmetics, food contact materials, medical devices; USFDA regulations; European Commission's FP7 NanoTEST project in 2012 [5]. The Organisation for Economic Co-operation and Development (OECD) is an inter-governmental organization with representatives of 38 countries in North and South America, Europe and the Asia and Pacific region. We have tried to briefly compile the guidelines of these various agencies in following Tables 2, 3, 4, and 5.

One important declaration by FDA is that it does not categorically judge all products containing nanomaterials or otherwise involving the use of nanotechnology as intrinsically benign or harmful. Rather, for all products (nanotechnology-derived or otherwise), FDA considers the characteristics of the product and its safety and effectiveness for its use. Berkeley, California is currently the only city in the United States to regulate nanotechnology through an Assembly Bill, 2006. Each

Table 2 European Union Nanomedicines Regulatory Coalition (7 Associations) Suggested Guidelines. Adapted from [18]

Regulatory concern	Steps suggested
For research and applications based on nanomedicines and nanosimilars	Assessment of all nanomedicines and nanosimilars by the European Medical Association (EMA) Centralised Regulatory Procedure. Development of harmonized protocols for regulators to correctly characterize diverse classes of nanomedicines. Establishment of a scientific consensus on definitions for nanomedicines across Europe.
For streamlining of regulatory procedures for nanomedicines and nanosimilars	Nanomedicines should have more stringent regulations than generic drugs. Same nanomedicines being sold under different brand names and getting approved by different routes. Hence, a consolidation of pharmacovigilance needs to be done. Revision of the EU Pharmaceutical Legislation is required to include an extended scope of mandatory and centralized procedures for defined nanomedicines. Conduction of regular educational workshops for scientists, policy makers, patients etc.

Table 3 OECD Guidelines [6, 30]

Issues of concern	Tests suggested
Multi-parametric testing accepted by all OECD members (MAD–Mutual Acceptance of Data)	Determination of physical and chemical characteristics of nanoparticles. Determination of effect of nanoparticles on biotic systems, using *in vitro* and *in vivo* assays Determination of environmental fate and behavior of nanoparticles Determination of ecotoxicity and biotoxicity Detailed health effects of nanoparticles in nanomedicine
Physical characterization of nanomaterials	Nano material name Degree of purity, known impurities or additives Basic morphology Description of surface chemistry (e.g. coating or modification) Known catalytic activity
Physico-chemical characterization of nanomaterials	Agglomeration/aggregation Water solubility Crystalline phase Crystalline size Dustiness TEM pictures Particle size distribution Specific surface area Zeta potential (Surface charge) Surface chemistry Photocatalytic activity Pour density Porosity Octanol water partition coefficient Redox potential Radical formation
Determination of material safety	Flammability Explosivity Incompatibility

Table 4 USFDA Regulations [9]

Health concern	Tests suggested
Stability and Biocompatibility of nanomedicine in presence of human plasma and blood	Determination of percentage of nanoparticles binding with plasma and serum proteins. Clearance and metabolism of nanomedicine via phagocytes is the routine *in vivo* mechanism. So *in vitro* exposure of cultured human phagocytes to nanomedicine is needed to evaluate its potential cytotoxicity.
For determination of Immunogenicity	ICH guideline S8 for Immunotoxicity needs to be followed
For determination of Immunotoxicity	Testing of several aspects of the immune response: Inducible proliferative response, Phagocytic activity, Respiratory burst of leukocytes, Natural killer cell activity, Production/ expression of cytokines
For determination of Risk Category for nanomedicines	Low clinical risk: For example, drug products (administered by oral, topical, or parenteral routes) containing nanomaterials that revert to their molecular constituents immediately after administration are likely to present low risk. Medium clinical risk: Expected to exhibit clinically significant changes in exposure, safety, and/or effectiveness relative to the native drug. For example, drug products containing non-targeted nanomaterials intended for systemic action are administered parentally. High clinical risk: Expected to exhibit clinically significant changes in exposure, safety, and/or effectiveness relative to the native drug. For example, drug products containing targeted nanomaterials intended for systemic action and that are administered intravenously.
For determination of metabolism of nanomedicines	Separate PK-PD studies need to be undertaken. Detailed Bioequivalence studies need to be undertaken.

Table 5 European Commission's FP7 NanoTEST Guidelines [5]

Health concern	Tests suggested
For nanoparticle toxicity determination	2–3 different cytotoxicity tests like the MTT, WST-1 and plating efficiency assays or relative growth activity Use of at least 2–3 representative cell lines Use of at least five Nanoparticle concentrations during assay protocol Inclusion of proper positive and negative controls
For determination of free radical release (if any) after use of nanomedicine under study	Use of 2–3 different assays for determination of oxidative stress
For determination of immunotoxicity	Testing of several aspects of the immune response: Inducible proliferative response, Phagocytic activity, Respiratory burst of leukocytes, Natural killer cell activity, Production/ expression of cytokines
For determination of genotoxicity	Use of modified comet assay for DNA damage (strand breaks as well as oxidised DNA lesions) Micronucleus detection assay Gene mutation test
Assays for effects of Nanoparticle after membrane transport and cellular uptake	Still not standardized

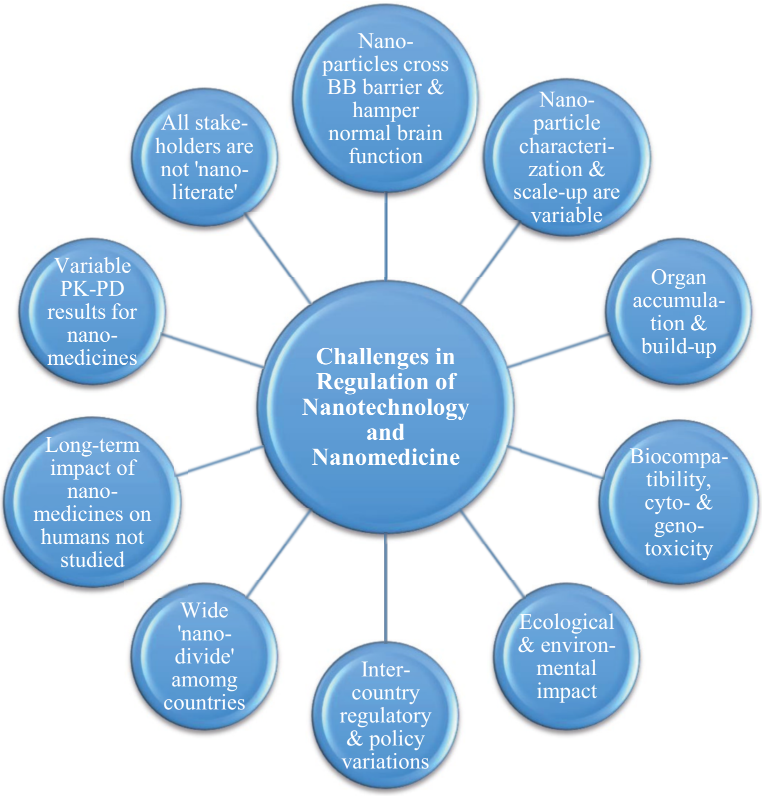
Challenges in Regulation of Nanotechnology and Nanomedicine
Nano-particles cross BB barrier & hamper normal brain function
Nano-particle characteri-zation & scale-up are variable
Organ accumula-tion & build-up
Biocompa-tibility, cyto- & geno-toxicity
Ecological & environ-mental impact
Inter-country regulatory & policy variations
Wide 'nano-divide' amomg countries
Long-term impact of nano-medicines on humans not studied
Variable PK-PD results for nano-medicines
All stake-holders are not 'nano-literate'

Fig. 2 Challenges in Regulation of Nanomaterial and Nanomedicine

nanomedicine manufacturer must contact FDA upfront to decide upon safety protocols for their particular medicine (Fig. 2).

The Basel Convention

The Basel Convention regulates the movement of hazardous waste across international borders. It came into effect globally in 1992. The convention's goal is to protect the environment by regulating and controlling the disposal of hazardous and other waste. The convention covers toxic, poisonous, explosive, corrosive, flammable, ecotoxic, and infectious wastes. Later in the year 2017–2018, nanomaterial waste was also included under the purview of this program. It further emphasized the need for United Nations Institute for Training and Research (UNITAR) and Organisation for Economic Co-operation and Development (OECD) to continue development and dissemination of international guidance and training materials related to safety of nanomaterials. It asked Strategic Approach to International Chemicals Management (SAICM) stakeholders to consider using the guidance for the Development of a National Nanotechnology Policy and Programme in their own countries but as of today, very few countries have developed thorough guidelines [41].

4 Social Implications of Nanotechnology and Nanomedicine

4.1 Public Perception

Individuals are continuously fed with social and news content through various platforms of media. Individuals are expected to be aware of different words of matter, even if they may/ not be sensitive towards it. The multitude of expanse of exposure to media on public has a direct impact on their opinion on different subjects. The say of individuals is documented through surveys, campaigns, and feedback mechanisms. The framework, presentation, intensity, tone and deliverance of a story can influence how the individuals think and feel about a topic. Public understanding of a nanomaterial—either in the form of medicine or otherwise is dependent on the education campaigns and awareness in general.

In a large-scale telephonic survey on levels of knowledge and attitude of the US population, 71% of respondents judged nanotechnology to be among the most valuable sources of health management in the twenty-first century [36]. A pilot survey among 23 US dermatologists evaluated their understanding and knowledge about nanotechnology [11]. The survey found that most of the respondents considered nanotechnology research to contribute in understanding (78.26%), diagnosis (73.91%) and treatment (78.26%) of skin diseases. Most respondents recognized that research was necessary to gauge safety of nanomaterial (86.96%). The delivery of nanomedicine is mediated via the medical doctors and hence their awareness is crucial to extend it to the patient community. An awareness study in Sri Lankan medical undergraduates' revealed that 86% of 80 respondents knew sizes of nanomaterials, 42% were aware of nanomedicine and 34% supported utility towards diagnosis and treatment [23]. The global public opinion about nanotechnology has been articulated in last two decades [28]. The analyses of the response of the public shows that the respondents used heuristics to achieve the conclusions/options for the survey, on questions on which they have less information [34, 35]. These heuristics have logic based on different practices, faith (practiced and non-practiced), recent information as sourced from various beliefs, systems of value, short-term information provided by several references [4, 17]. This makes heuristics as a variable that changes across geographies and time. Hence a reproducibility of responses is impossible.

Media Influence on Public Opinion

Public opinion is highly influenced by the media. Traditional media of print like newspaper and magazines have consistently provided information to the masses. Other forms of media involving mass communications mediated by newsroom, bloggers, vloggers, commentators, podcasters etc., provide a perspective to the information, based on which, the opinion can be constructed in a way the narration is built. Selection of the theme, the knowledge of the commentators and flow of discussion that is presented and debated at the prime time of news viewership influence the public mood and opinion. Media productions houses form the primary sources of news and information for individuals as they bring them access to large information that is otherwise not accessible in general. The expanse of citizens becoming a local reporter is limited to timely availability of suitable devices, choice of media platform and awareness to other individuals. Such cases usually arise in places of conflict where media and reporting are allowed. It is up to the media actors to select the framing of a theme on which an opinion may be developed. This is itself based on perception of public utility and interest, and if it merits publication criteria [7]. This is juxtaposed to the acceptance of the published stories by the public and hence the success of the media house. This creates a bias based on the profitability or salability of certain themes. An imposition of 'matters of public interest' may be created. This results in putting a continued emphasis on various related events and putting feedback from the public in the form of random survey. In matters of reporting, however, a neutral approach is best suited. The media is hence a major contributor to developing and eventually influencing the opinion of the masses [33]. In a study focused on public opinion on education in Portugal during a period of 2001–2017, the small number of themes that gained more importance at specific moments, closely followed the themes in the Organisation for Economic Co-operation and Development (OECD) agenda [33].

The media has become a fulcrum of public service with public agenda. In matters of health and technology, the discussions and inputs from technical personnel becomes critical. Public institutions are better versed to bring forth an overall idea of the technology in comparison to for-profit organizations that may bring a biased presentation. Government policies that promote certain sectors for growth is contributed by the political will. Considering the influence of government bodies on media platforms, the media may become the tool to highlight the state practices. Media may become the forum for development of policy decisions as well [7]. In the case of Nanotechnology and Nanomedicine, media has power to educate the public, identify the key questions that the public asks and highlight the analysis for different stakeholders. The domain of nanotechnology has been new and evolving since the last few decades and media becomes the educational platform.

Public Awareness Campaigns

Public participation has five levels of engagement, where the lowest three levels (inform, consult and involve) are preferred over the two higher levels (collaborate and empower). Public awareness campaigns are necessary to inform the masses. Government policies and schemes to promote Nanotechnology are key to effective transition and implementation. The direct implication of Nanomedicine is the healthcare sector. The public should be informed about the current resource materials, the risks involved in using Nanomedicine, and the choices they have. A well-informed community is empowered to have suitable actions. In a recent review on public awareness on climate change adaptation policies, Khatibi et al. [20]

identified communication to be a significant factor in improving knowledge and awareness of the masses. Active engagement with the community aids in awareness and policy development. In the absence of information and knowledge, the public becomes disinterested even in passive engagement. An online questionnaire based survey about the air pollution in Muscat, Oman was performed between February and May of 2020 [2]. The majority of the respondents were aware of dangers of air pollution to their health. Social media was a primary source of access to information regarding air pollution in Muscat. Many were willing to change their mode of transportation behavior (from private to public) to reduce air pollution. An early behavioral change towards acceptance of newly introduced Nanomedicine can happen when the public is aware of and well involved in the policies being implemented. A negative attitude is usually associated with less nanotechnology-related knowledge [19]. Various interdependent factors can differentiate the risk-benefit perception of nanotechnology. Further, respondents having a negative attitude are less likely to be asking for further information about the subjects of nanotechnology/nanomedicine. The communities in the developed and the developing countries tend to have a positive attitude towards nanotechnology [31]. Considering that the public opinion is influenced by cultural practices, religious beliefs, trust in science, and media exposure, the demographics does not depict a uniform public attitude about nanotechnology. The international NanOpinion project, focused on raising public awareness about nanotechnology, had a key takeaways [24]. The project had organized outreach events in 44 different locations, across 26 cities in 18 countries. The massive activity connected with approximately 14,400 participants. The facilitators showed nanotechnology products, performed and demonstrated simple experiments out of the ‘nano schoolbox’ kit for schools. Many of the project outlets had opinion boards and feedback was taken from visitors.

4.2 Societal Impact Assessment

Research on development of nanotechnology and nanomedicine may be carried out by an individual or a group of research laboratories. However, their effect as applied on healthcare system requires a broader scale of assessment. The impact of a policy or development plan over various domains of an individual (work-related, and personal interactions), cultural practices, political systems, health and general well-being can be determined by Social Impact Assessment (SIA). SIA is a method to determine, predict, and estimate the known and unknown social effects of proposed policy or a project [3]. The process allows mitigation of any unintended or negative impacts on the affected community. SIA permits the upholding of principles of social sustainability for any proposed policy.

Methodologies for Assessing Societal Impact

Tools that can measure the social impact are of utmost necessity for the entire ecosystem. Government and private research funding agencies look at the long-term societal benefits to the projects funded through them. The return of such an investment is largely seen through the differences made in the lives of public at large. Their positive impact proves their legitimacy and worth. SIA is an important tool for any policy for public. As such, the funding institutions require suitable measures to determine the worthiness of any proposal. Sorensen et al. [39] developed a quantifiable approach for occupational health and safety. The approach termed *the Societal Impact Instrument: Occupational Health and Safety Research* (SII:OHSR), creates a collective measure of societal impact for the research institution and each research program. Reed et al. [32] presented five types of impact evaluation design: (i) experimental and statistical methods; (ii) textual, oral and arts-based methods; (iii) systems analysis methods; (iv) indicator-based approaches; and (v) evidence synthesis approaches. These are some of the recent methods for evaluating projects and policies.

Case Studies in Societal Impact Assessment

Different countries now follow their defined guidelines to achieve SIA. Horizon Europe has been the most developed programme within the EU framework programme [8]. Governments are incentivizing the generation of impact through conditions directly associated with research funding and their evaluations. The UK's Research Excellence Framework (REF) incorporated an evaluation of impact using case studies based on their importance and public reach. Swedish Research Council Strategic Research Centres and Hong Kong's University Grants Committee are required to submit the impact case studies for assessment on lines of UK REF [42]. The Spanish National Commission on the Evaluation of Research Performance (CNAI) gives a financial incentives to investigators who provide 'evidence of impact and influence' of their research 'on social and economic matters' as a part of their six-yearly individual research presentation [14]. In USA, the National Science Foundation, National Institute of Health, US Department of Agriculture, and US Environmental Protection Authority established a repository of records for gauging the impact of federal research and development investment [25].

4.3 Ethical and Social Convergence in Nanotechnology and Nanomedicine

The inevitable pace of development of innovations in nanotechnology and nanomedicine brings significance to the concerns of the unknown impacts the new developments present to human health, the environment and society at large. The physico-chemical and biological properties of nanomaterials are expected to define parameters of consideration of it effect on nature and human health. The development of nanomaterials in the lab to its application to the body involves the co-development of research and policies. In absence or delay of close interactions, the policy will impact the research developed and the course correction may lead to loss of time and resources. Early congregations to study the negative effects of nanoparticles were discussed in the U.S. Congress way back in 2003 where studies dedicated on the ethics were to be performed at the American Nanotechnology Preparedness Center [22]. The expanse of nanobiotechnology led to involvement of multiple agencies apart from U.S. National Institutes of Health (NIH) and Occupational Safety and Health Administration (OSHA) in the oversight discussion and programme [29]. The Food and Drug Administration (FDA), Environmental Protection Agency (EPA), and Department of Agriculture (USDA) were also involved in the study. The authors Paradise et al., presented a new way of evaluating oversight using a range of quantitative and qualitative analysis to evaluate selected cases studies,. The qualitative assessment involved the criteria of development, attributes, extent of change and outcomes for the Genetically Engineering Organisms, Drugs, Devices, Workplace chemical and Gene Therapy. Hall used classical principles used for medical ethics of Beneficence, Non-Maleficence, Respect, and Justice in the context of nanotechnology [16]. In the current context, Beneficence is the obligation of providing the utmost good to the people through the use of nanotechnology; Non-Maleficence is principled to prevent any harm through nanotechnology. An unintended harm has to be therefore addressed by various agencies before the nanomaterials are made available. Respect is centered around the autonomy, followed by informed consent, the privacy and performance enhancement [16]. Justice relates to the belief that equitable access to health though nanotechnology should be provided.

4.4 Social Responsibility of Nanotechnology Stakeholders

Social responsibility of nanotechnology allows for defining the agenda required for imparting the awareness in the society. The society can be divided into (i) intended recipients—the consumers of nanotechnology or nanomedicine products and (ii) unintended and exposed individuals—the regulators involved in quality check, distribution and delivery individuals like doctors and technical support staff, the funding agency, organizations & individuals working near the waste produced from the nanomaterial producing industry and waste produced from the intended recipients among others. The society and the environment form the ultimate stakeholders for the research and development in the nanotechnology. Considering that the Nanotechnology and Nanomedicine is majorly in research domain, it follows the classical method of research-development-innovation. Thus, in the current context, the social responsibility has both direct and indirect influences on the society as a whole.

5 The Role of Stakeholders

The current attention that is being received by nanotechnology is in growth phase. A decade back, USA allocated a budget of USD 2.1 billion in 2012, while it had spent USD 16.5 billion since 2001 for nanotechnological research [13]. The budget has been spent on the fields of environment, health and defense industries (USD 575 billion). A smaller fraction, of worth USD 390 billion was spent on the research for ethical, legal and social effects of nanotechnology. The UNESCO's International Bioethics Committee (IBC) is an independent body comprising of 36 experts from various domains and of different countries. UNESCO has carried out studies to identify and determine the advantages and disadvantages about nanotechnology [40]. Over many years of IBC formation, the committee has discussed various aspects that are of concern and require a global perspective due to its sheer enormity [12]. Various countries have established their own strategies to manage various aspects of nanomedicine. For example, the US government started the National Nanotechnology Initiative in 2000 to encourage the responsible R&D of nanotechnology (National Nanotechnology Initiative, 2021). At the state level, the Arizona State University and the University of California, Santa Barbara, have dedicated centers that have their research focused on the legal and ethical issues (Centers for Nanotechnology in Society, Arizona State University, 2021; UCSB, CNS, 2020). South Korea established their National Nanotechnology Policy Center in 2010 (National Nanotechnology Policy Center, 2010), while the European Union's Horizon 2020 has proceeded nano-related programs since 2014 (European Union, 2020). In India, Department of Biotechnology (DBT), Government of India broadly recognizes three types of nanomedicines, namely, nanocarriers for drug delivery, nanosuspensions and nanoparticles for bioimaging. To promote nanotechnology, Government of India launched a Mission on Nano Science and Technology ('Nano Mission') in May 2007 which succeeded the Nano Science and Technology Initiative (NSTI) of Department of Science and Technology (DST), 2001. This "Nano Mission' and CKMNT, Secunderabad have published a detailed document regarding use of nanotechnology and nanomedicine in India (2012). This document is available in public domain (https://dst.gov.in/sites/default/files/Draft-Guidelines.pdf, accessed on 25 Apr. 2024). Similarly, the DBT published its own guidelines on 'Nanopharmaceuticals (Nanomedicines) in India" in 2019 and they are available in public domain (https://dbtindia.gov.in/sites/default/files/uploadfiles/Guidelines_For_Evaluation_of_Nanopharmaceuticals_in_India_24.10.19.pdf, accessed on 25 Apr. 2024). The guidelines apply to the nano-pharmaceuticals either as finished formulation or as API of a new molecule or an already approved molecule with altered nano- scale dimensions, properties or phenomenon associated with the application of nanotechnology, intended to be used for treatment, *in vivo* diagnosis, mitigation, cure or prevention of diseases and disorders in humans. A major category of risk related areas come under the responsibility of Ministry of Environment and Forest (MOEF) but none of the MOEF acts and legislation explicitly identify nanoparticles as a potential hazard. TERI is the only NGO that drew most attention to environmental, health and safety issues, and ethical, legal and social issues related to nanotechnology. Due to rising demand and increased focus on research and development, the nanotechnology market in India is poised to grow with a compound annual growth rate of 19.1% in the next decade. Presently in India, 21 clinical trials of nanomedicines are underway. Ranbaxy's 'Volini Nanogel' has been marketed; so regulatory guidelines need to be formulated quickly to mitigate the hazardous outcome of any nanotechnology-based drug. The latest entrant in the market is the 'USENSe test developed by Module Innovations that detects urinary tract infections within fifteen minutes, by combining nanotech and biotech (https://moduleinnovations.com/, accessed on 14 Apr. 2024). The company uses one-time and reusable nanofiber strips that can specifically detect pathogens causing UTI.

6 Future Trends and Recommendations

The domain of nanomedicine is not confined to one scientific field or industrial sector, and nanotechnology is expected to have an impact that crosses many existing disciplinary and institutional boundaries. The guideline for preparation for nanomedicines is certainly not an easy task due to the lack of extensive and deep scientific knowledge as well as tools and techniques. Technology and development are intricately related. Nanomedicine development needs high funding. This is especially complex in the current world where funds and technological disparities are wide. There is a distinct divide between developed nations and poor nations in terms of technology development and translation. Thus, nanotech can lead to new social divides and non-equitable resource allocations. We must strike a balance between nanomedicine innovation and the responsibility to ensure that it would be used in an ethical, fair and beneficial manner for society as a whole:

- Ethical considerations: It is crucial to prioritize ethical considerations in the development and deployment of fast-developing nanomedicine. Ethical frameworks and guidelines must be established to guide the decision-making process. It is imperative to protect the interests of technologically limited sections of the global society.
- Inclusive and fair access: Nanomedicine research should be designed and implemented to promote inclusivity and equal access for all members of the society.
- Collaboration with stakeholders: Collaboration between scientists, policymakers, and various stakeholders right from the beginning helps to ensure responsible and ethical use of nanotechnology. Continuous open dialogue and feedback collection from diverse societal strata will help to identify potential risks and to address societal concerns.
- Regulation and governance: Governments and regulatory bodies need to follow a stringent time-based approach to establish policies, standards, and regulations to govern the use of nanotechnology. A collaboration between industry and regu-

latory bodies to balance innovation and responsible use will ensure compliance and accountability.
- Regulation from a research perspective: Issues related to the framing of a globally acceptable definition of nanoparticles and their production modalities such as the infrastructure, trained manpower, unanimous quality control parameters, clear guidelines about occupational hazards and associated health risks, nanotoxicological and PK/PD studies of nanodrugs as well as nanocarriers need to be addressed urgently.
- Continuous monitoring and evaluation: Regular monitoring, evaluation of the impact of nanotechnology and agile course correction in case of unintended consequences or negative effects is the need of the hour.
- Ethical design principles: Incorporating ethical design principles, such as user-centric design, can help ensure that nanotechnology is developed with ethical considerations from the early stages. Diverse perspectives and the potential impacts on individuals and communities need to be studied in detail.
- Public awareness and education: Promoting public awareness and education about the responsible use of nanotechnology can empower individuals to understand and navigate the potential implications. This can include awareness initiatives via print and social media, public lectures by academicians and industries and policy makers.

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Exploring Nanomedicine Ventures and Latest Developments in Clinical Trials

Umesh D. Patil

1 Introduction

1.1 Brief Overview of Nanomedicine

Nanomedicine, born at the nexus of nanotechnology and medicine, stands as a testament to the remarkable synergy between scientific disciplines. Its inception traces back to the late twentieth century, spurred by breakthroughs in nanomaterial synthesis and characterization. The field emerged from the recognition that materials at the nanoscale exhibit unique properties, such as high surface area-to-volume ratios and tunable surface chemistry, which render them promising candidates for biomedical applications [1–5].

The roots of nanomedicine can be traced to seminal works in the 1950s, including Richard Feynman's famous lecture on "There's Plenty of Room at the Bottom," which laid the conceptual groundwork for manipulating matter at the atomic and molecular levels. However, it wasn't until the 1990s that nanomedicine began to take shape as a distinct field, with the pioneering work of researchers like Robert Langer and Nicholas Peppas, who explored the potential of nanoparticles for drug delivery and tissue engineering [6, 7].

Since then, nanomedicine has experienced exponential growth, driven by advances in nanomaterial synthesis, nanofabrication techniques, and our understanding of biological systems at the nanoscale. The field has expanded to encompass a wide range of applications, including targeted drug delivery, imaging contrast agents, biosensors, regenerative medicine, and theranostics [8–10].

Today, nanomedicine holds immense promise for revolutionizing healthcare by offering tailored solutions for disease diagnosis, treatment, and monitoring.

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Nanoparticle-based drug delivery systems enable precise control over drug release kinetics and tissue targeting, minimizing side effects and improving therapeutic outcomes [11–13]. Nanoscale imaging agents provide unprecedented resolution and sensitivity for early disease detection and monitoring treatment responses.

Nanomedicine represents a convergence of cutting-edge science, engineering, and medicine, poised to transform the landscape of healthcare delivery. As research continues to push the boundaries of nanotechnology and our understanding of biological processes deepens, the future of nanomedicine holds boundless opportunities for innovation and impact.

1.2 Importance of Clinical Trials in Validating Nanomedicine Applications

Clinical trials serve as the cornerstone in the journey of nanomedicine from laboratory discovery to clinical implementation, offering critical insights into safety, efficacy, and therapeutic outcomes. These trials are imperative for bridging the gap between preclinical research and real-world healthcare settings, ensuring that nanomedicine interventions meet rigorous standards of scientific scrutiny and regulatory approval. Central to the advancement of nanomedicine is the rigorous evaluation of safety, efficacy, and therapeutic outcomes through well-designed clinical trials. Clinical trials serve as the cornerstone for validating nanomedicine applications, providing critical evidence for regulatory approval, clinical adoption, and translation into standard clinical practice [14].

Clinical trials enable researchers to assess the pharmacokinetics, biodistribution, and biocompatibility of nanomedicine formulations in human subjects, elucidating their behavior in vivo and informing dosing regimens, administration routes, and patient selection criteria. By systematically evaluating nanomedicine products in controlled clinical settings, researchers can quantify therapeutic effects, monitor adverse events, and optimize treatment protocols to maximize efficacy and minimize risks [15–17].

Clinical trials play a pivotal role in establishing the comparative effectiveness of nanomedicine therapies versus standard of care or existing treatment modalities. Comparative studies provide insights into the added value of nanotechnology-based interventions, such as improved targeting, reduced side effects, enhanced therapeutic index, and prolonged survival rates, guiding clinical decision-making and healthcare resource allocation.

1.3 Significance of Exploring Ventures in Nanomedicine

Venturing into the realm of nanomedicine holds immense significance for healthcare innovation, economic growth, and societal well-being. Nanomedicine ventures encompass a broad spectrum of activities, including academic research, industrial

R&D, technology transfer, startup incubation, and commercialization initiatives, all aimed at translating scientific discoveries into tangible healthcare solutions [18, 19].

From an economic perspective, nanomedicine ventures drive investment, job creation, and economic growth by fostering innovation, entrepreneurship, and technology commercialization. Startups and SMEs play a pivotal role in bringing nanomedicine products to market, capitalizing on scientific breakthroughs and addressing unmet medical needs in diverse therapeutic areas, including oncology, neurology, cardiology, and infectious diseases. Exploring ventures in nanomedicine facilitates knowledge exchange, collaboration, and capacity-building across academia, industry, and government sectors, catalyzing interdisciplinary research, talent development, and infrastructure investment. Collaborative innovation ecosystems provide fertile ground for cross-disciplinary partnerships, technology transfer, and translational research, accelerating the pace of discovery and commercialization in nanomedicine [20, 21].

The significance of exploring ventures in nanomedicine lies in its potential to transform healthcare delivery, improve patient outcomes, and address global health challenges. By fostering innovation, collaboration, and entrepreneurship, nanomedicine ventures pave the way for a future where precision medicine, personalized diagnostics, and targeted therapies are the norm, offering hope and healing to patients worldwide.

2 Fundamentals of Nanomedicine Ventures

2.1 Definition and Scope of Nanomedicine Ventures

Nanomedicine ventures encapsulate a dynamic intersection of scientific exploration, entrepreneurial endeavor, and healthcare innovation, fuelled by the transformative potential of nanotechnology. Extending patient care and solving complicated biological problems are the fundamental goals of nanomedicine initiatives, which aim to use the distinctive characteristics of nanoparticles and nanomaterials [19, 22].

The scope of nanomedicine ventures is multifaceted, encompassing a spectrum of activities ranging from fundamental research to commercialization and clinical translation. At the foundational level, nanomedicine ventures entail interdisciplinary collaborations between scientists, engineers, and clinicians to develop novel nanomaterials, drug delivery systems, and diagnostic platforms. Nanoparticles with specific features and functions are the goal of these efforts, which use concepts from engineering, biology, chemistry, and physics.

Moving beyond the laboratory bench, nanomedicine ventures extend into translational research, where promising nanotechnologies are evaluated in preclinical models and optimized for clinical applications. This phase involves rigorous testing of nanomedicine formulations for safety, efficacy, and biocompatibility, paving the way for regulatory approval and clinical trials.

The entrepreneurial aspect of nanomedicine ventures comes to the fore in the realm of technology transfer, startup incubation, and industry partnerships. Startups and small-to-medium-sized enterprises (SMEs) play a pivotal role in driving innovation and commercialization, leveraging academic research and intellectual property to develop nanomedicine products and bring them to market [23–25]. These ventures navigate the complex landscape of regulatory compliance, intellectual property protection, and market access, with the aim of delivering impactful solutions to patients and healthcare providers.

Nanomedicine ventures extend beyond traditional boundaries to encompass global collaborations, policy advocacy, and societal engagement. International partnerships facilitate knowledge exchange, resource sharing, and capacity building, fostering innovation and accelerating the pace of nanomedicine development worldwide. Furthermore, advocacy efforts seek to raise awareness of nanomedicine's potential benefits and address ethical, legal, and social implications, ensuring responsible and equitable deployment of nanotechnology in healthcare [26, 27].

In a nutshell, nanomedicine ventures represent a convergence of scientific inquiry, technological innovation, and entrepreneurial spirit, poised to revolutionize healthcare delivery and improve patient outcomes. By embracing interdisciplinary collaboration, translational research, and entrepreneurial drive, nanomedicine ventures hold the promise of unlocking new frontiers in precision medicine, personalized diagnostics, and targeted therapies, ushering in a new era of healthcare innovation and impact.

2.2 Role of Interdisciplinary Collaboration in Nanomedicine Ventures

Interdisciplinary collaboration lies at the core of nanomedicine ventures, serving as the driving force behind the translation of nanotechnology-enabled solutions from bench to bedside. By integrating insights from materials science, biology, engineering, and clinical medicine, interdisciplinary teams forge synergistic partnerships that transcend disciplinary boundaries, thus enhancing the robustness and relevance of their endeavours [26, 27].

Materials scientists play a pivotal role in the design and synthesis of nanomaterials tailored to specific biomedical applications, leveraging their expertise in nanoscale fabrication techniques and material characterization methods. Concurrently, biologists and pharmacologists contribute invaluable insights into the biological interactions of nanomaterials within living systems, elucidating mechanisms of cellular uptake, biodistribution, and therapeutic efficacy [28].

Engineers bring their proficiency in microfabrication, nanofabrication, and bioengineering to bear on the development of advanced nanoscale platforms for diagnostics, imaging, and therapeutics [29–31]. Through collaborative efforts, clinicians provide critical input on clinical needs, guiding the selection of target diseases, endpoints, and patient populations for preclinical and clinical studies (Table 1).

Industry partners play a crucial role in facilitating technology transfer, scale-up, and commercialization of nanomedicine innovations, thereby bridging the translational gap between research and market adoption. By fostering a culture of collaboration and knowledge exchange, interdisciplinary teams harness the collective intellect and resources of diverse stakeholders to overcome complex challenges and realize the full potential of nanomedicine.

2.3 Overview of Key Players and Institutions Involved in Nanomedicine Research and Development

The landscape of nanomedicine research and development is characterized by a rich tapestry of academic institutions, research organizations, government agencies, industry partners, and healthcare providers, all united by a shared commitment to advancing the frontiers of biomedical science and technology.

At the forefront of nanomedicine innovation are leading academic institutions and research centers, which serve as incubators for groundbreaking discoveries and transformative technologies. Institutions such as MIT, Stanford University, and the National Institutes of Health (NIH) foster collaborative research environments, driving interdisciplinary initiatives aimed at addressing pressing medical challenges [32, 33].

Complementing academic efforts are research organizations and consortia dedicated to advancing nanomedicine through funding, infrastructure, and collaborative initiatives. Organizations such as the National Nanotechnology Initiative (NNI) and the European Commission’s Horizon 2020 program provide critical support for interdisciplinary research projects, facilitating technology transfer and commercialization efforts [34].

Government agencies, including regulatory bodies such as the FDA, EMA, and Health Canada, play a pivotal role in ensuring the safety, efficacy, and quality of nanomedicine products through rigorous oversight and regulatory guidance [35–37]. These agencies provide essential frameworks for preclinical testing, clinical trial design, and market approval, thereby safeguarding public health while fostering innovation and investment in nanomedicine.

Industry partners, ranging from pharmaceutical companies to biotechnology startups and medical device manufacturers, drive the translation of nanomedicine research into commercial products and therapies. Companies such as Pfizer, Johnson & Johnson, and Novartis invest in research and development initiatives, forge strategic partnerships with academic institutions, and navigate regulatory pathways to bring novel nanomedicine products to market [38, 39].

Healthcare providers, including hospitals, clinics, and medical centers, serve as vital conduits for implementing and evaluating nanomedicine technologies in clinical practice. Through clinical trials, patient care, and technology assessment, healthcare providers contribute to the validation, adoption, and optimization of nanomedicine interventions, thereby shaping the future of healthcare delivery.

Table 1 Clinical trials demonstrate the versatility and potential of nanotechnology in addressing various diseases and conditions

Sr. No.	Title	Status	Study Results	Conditions	Interventions	Locations
1	Mechanism of Hypersensitivity Reactions to Iron Nanomedicines	Completed	No Results Available	Iron-deficiency	Behavioral: hypersensitivity reaction	University Hospital, Basel, Switzerland
2	BIIR Gene to Manage Heart Allograft Patients	Completed	No Results Available	Cardiac Transplant Failure		Baylor University Medical Center, Dallas, Texas, United States
3	Single DermaVir Immunization in HIV-1 Infected Patients on HAART	Completed	Has Results	HIV Infection	Biological: DermaVir Drug: HAART	Saint Laszlo Hospital, Budapest, Hungary
4	Repeated DermaVir Immunizations in HIV-1 Infected Treatment-naïve Patients	Completed	No Results Available	HIV Infection	Biological: DermaVir Biological: Placebo	ifi-Medizin GmbH at the Asklepios Klinik St. Georg, Hamburg, Germany ICH Grindel, Hamburg, Germany University Medical Center Hamburg-Eppendorf, Hamburg, Germany
5	31P-MRS Imaging to Assess the Effects of CNM-Au8 on Impaired Neuronal Redox State in Parkinson's Disease	Completed	No Results Available	Parkinson’s Disease	Drug: Gold Nanocrystals	UT Southwestern, Dallas, Texas, United States

(continued)

Table 1 (continued)

Sr. No.	Title	Status	Study Results	Conditions	Interventions	Locations
6	Antiretroviral-Sparing Concept With HIV-specific T Cell Precursors With High Proliferative Capacity (PHPC)	Completed	No Results Available	HIV Infection	Biological: DermaVir Biological: Placebo Drug: HAART	IRCCS Policlinico S. Matteo, Pavia, Italy
7	Neurotoxicity Characterization Study of Nab-paclitaxel Versus Conventional Paclitaxel in Metastatic Breast Cancer	Completed	No Results Available	Breast Cancer	Drug: Paclitaxel 80 mg/m^2 Drug: Nab-paclitaxel 100 mg/m^2 days 1, 8 and 15 Drug: Nab-paclitaxel 150 mg/m^2 days 1, 8 and 15 Drug: Nab-paclitaxel 150 mg/m^2 days 1 and 15	Hospital Universitario Del Sureste, Arganda del Rey, Madrid, Spain Hospital Universitario de Fuenlabrada, Fuenlabrada, Madrid, Spain Hospital Universitario de Getafe, Getafe, Madrid, Spain Hospital Universitario Severo Ochoa, Leganés, Madrid, Spain Hospital Universitario Puerta de Hierro Majadahonda, Majadahonda, Madrid, Spain Hospital Universitario Infanta Cristina, Parla, Madrid, Spain Hospital Universitario Infanta Leonor, Madrid, Spain Hospital Ramón Y Cajal, Madrid, Spain Hospital Clínico San Carlos, Madrid, Spain Hospital 12 de Octubre, Madrid, Spain

(continued)

Table 1 (continued)

Sr. No.	Title	Status	Study Results	Conditions	Interventions	Locations
8	A Multi-Center, Open-Label Long-Term Extension Study of CNM-Au8 In Patients With Stable Relapsing Multiple Sclerosis	Completed	No Results Available	Relapsing Multiple Sclerosis	Drug: CNM-Au8	Sydney Brain Mind Centre, Camperdown, New South Wales, Australia John Hunter Hospital, New Lambton Heights, New South Wales, Australia Princess Alexandra Hospital, Woolloongabba, Queensland, Australia Menzies Institute for Medical Research, Hobart, TAZ, Australia The Alfred Centre Department of Neuroscience, Melbourne, Victoria, Australia
9	Study of ZnAg Liquid Solution to Treat COVID-19 Symptomatic Participants	Completed	No Results Available	Covid19	Drug: CNM-ZnAg Drug: Placebo	PROCAPE, Santo Amaro, Recife, Brazil Projeto Praca Onze, Centro, Rio De Janeiro, Brazil IBPClin, Gloria, Rio De Janeiro, Brazil Casa de Saúde, Campinas, Sau Paulo, Brazil IPECC, Sao Paulo, State of Sau Paulo, Brazil

(continued)

Table 1 (continued)

Sr. No.	Title	Status	Study Results	Conditions	Interventions	Locations
10	Therapeutic Nanocatalysis to Slow Disease Progression of Amyotrophic Lateral Sclerosis (ALS)	Completed	No Results Available	Amyotrophic Lateral Sclerosis	Drug: CNM-Au8 Drug: Placebo	University of Sydney Brain and Mind Centre, Sydney, New South Wales, Australia Westmead Hospital, Sydney, New South Wales, Australia
11	A Phase I SAD and MAD Clinical Trial of CNM-Au8 in Healthy Male and Female Volunteers	Completed	No Results Available	Healthy Volunteers—Male and Female	Other: CNM-Au8 Other: Placebo	Centre for Human Drug Research (CHDR), Leiden, Netherlands
12	HEALEY ALS Platform Trial—Regimen C CNM-Au8	Completed	Has Results	Amyotrophic Lateral Sclerosis	Drug: CNM-Au8 Drug: Matching Placebo	Healey Center for ALS at Mass General, Boston, Massachusetts, United States

Data gathered from https://classic.clinicaltrials.gov

The field of nanomedicine is propelled by a vibrant ecosystem of stakeholders, each playing a distinctive and indispensable role in driving innovation, translation, and adoption of nanotechnology-enabled solutions for healthcare. By fostering collaboration and synergy across academia, industry, government, and healthcare sectors, these stakeholders collectively contribute to the advancement of nanomedicine and its transformative impact on patient care and public health.

3 Nanomedicine Innovations

3.1 Nanoparticle-Based Drug Delivery Systems

Design Principles and Engineering Considerations

Nanoparticle-based drug delivery systems represent a groundbreaking approach to enhancing the efficacy and safety of therapeutic interventions. These systems are meticulously engineered to encapsulate therapeutic agents within nanoscale carriers, facilitating their targeted delivery to specific tissues or cells while minimizing off-target effects. The design of nanoparticle-based drug delivery systems involves careful consideration of various factors, including nanoparticle composition, size, shape, surface properties, and payload loading capacity [40, 41].

Nanoparticles can be fabricated from a diverse range of materials, such as lipids, polymers, metals, and inorganic nanoparticles, each offering unique advantages and challenges. For instance, liposomal nanoparticles are composed of lipid bilayers and offer excellent biocompatibility and drug encapsulation efficiency. Polymeric nanoparticles, on the other hand, provide versatility in terms of surface modification and controlled drug release kinetics [42, 43].

Engineering considerations in nanoparticle design include optimizing nanoparticle size and surface properties to achieve desired pharmacokinetics and biodistribution profiles. Smaller nanoparticles can penetrate deep into tissues and exhibit prolonged circulation times in the bloodstream, while surface modifications with targeting ligands or stealth coatings can enhance nanoparticle specificity and evade immune recognition.

Furthermore, payload loading strategies, such as encapsulation, adsorption, or conjugation, play a crucial role in ensuring efficient drug delivery and release kinetics. Strategies such as stimuli-responsive drug release mechanisms enable triggered drug release in response to specific environmental cues, such as pH, temperature, or enzymatic activity, enhancing therapeutic efficacy while minimizing systemic toxicity [44–47].

Targeting Strategies for Specific Diseases

One of the key advantages of nanoparticle-based drug delivery systems is their ability to target diseased tissues or cells with high precision, thereby maximizing therapeutic efficacy and minimizing side effects [48, 49]. Targeting strategies exploit molecular signatures or physiological characteristics unique to diseased tissues, enabling selective nanoparticle accumulation at the target site.

Passive targeting relies on the enhanced permeability and retention (EPR) effect, which exploits the leaky vasculature and impaired lymphatic drainage commonly associated with tumors. Nanoparticles with appropriate size and surface properties can extravasate from leaky blood vessels and accumulate within the tumor microenvironment, enhancing drug delivery to cancer cells while minimizing exposure to healthy tissues [50].

Active targeting strategies involve the incorporation of targeting ligands, such as antibodies, peptides, or small molecules, onto the surface of nanoparticles. These ligands selectively bind to receptors overexpressed on the surface of diseased cells, facilitating receptor-mediated endocytosis and internalization of nanoparticles into target cells [51, 52]. This approach enables precise targeting of a wide range of diseases, including cancer, inflammatory disorders, and infectious diseases.

Overall, nanoparticle-based drug delivery systems represent a versatile platform for the development of targeted therapies with enhanced therapeutic efficacy and reduced systemic toxicity. By harnessing the principles of nanotechnology, researchers continue to refine and optimize these systems to address the unmet medical needs of patients worldwide.

3.2 Theranostic Nanoparticles for Simultaneous Diagnosis and Therapy

Theranostic nanoparticles represent a paradigm-shifting approach to personalized medicine, integrating diagnostic and therapeutic functionalities within a single nanoscale platform. These multifunctional nanoparticles enable real-time monitoring of disease progression while simultaneously delivering therapeutic agents to the target site, offering unparalleled opportunities for precision medicine and personalized therapy [53].

The design of theranostic nanoparticles involves the integration of diagnostic modalities, such as imaging agents or biosensors, with therapeutic payloads, such as drugs or nucleic acids. This integration enables simultaneous imaging of disease biomarkers and targeted drug delivery, facilitating early detection, monitoring of treatment response, and personalized treatment optimization [53].

Nanoparticle-based imaging agents, such as fluorescent dyes, quantum dots, or magnetic nanoparticles, enable high-resolution imaging of diseased tissues or cells with exquisite sensitivity and specificity. These imaging agents can be conjugated to targeting ligands to facilitate targeted imaging of specific biomarkers, enabling noninvasive detection and localization of disease sites [54–58].

Concurrently, therapeutic payloads, such as chemotherapeutic drugs, siRNAs, or therapeutic peptides, are encapsulated within or conjugated to the surface of nanoparticles [5, 59]. These payloads are designed to selectively accumulate within diseased tissues or cells, guided by targeting ligands or passive targeting mechanisms, thereby maximizing therapeutic efficacy while minimizing systemic toxicity.

Theranostic nanoparticles hold immense promise for a wide range of applications, including cancer therapy, cardiovascular disease management, and infectious disease diagnostics. By enabling real-time monitoring of treatment response and disease progression, these nanoparticles empower clinicians to tailor treatment regimens to individual patient needs, optimizing therapeutic outcomes and improving patient outcomes.

3.3 Nanoscale Imaging Agents for Improved Diagnostics

Nanoscale imaging agents represent a transformative approach to diagnostic imaging, offering enhanced sensitivity, resolution, and multiplexing capabilities compared to traditional imaging modalities. These agents leverage the unique optical, magnetic, or acoustic properties of nanomaterials to enable high-resolution imaging of biological structures and processes at the molecular level.

Fluorescent nanoparticles, such as quantum dots or fluorescent proteins, offer exceptional brightness and photostability, making them ideal candidates for fluorescence imaging of cells and tissues [60]. These nanoparticles can be functionalized with targeting ligands to enable specific imaging of disease biomarkers or molecular processes, providing valuable insights into disease pathology and progression.

Magnetic nanoparticles, such as iron oxide nanoparticles or gadolinium-based contrast agents, are widely used for magnetic resonance imaging (MRI) due to their ability to generate strong contrast enhancement [61]. These nanoparticles can be engineered to target specific tissues or cells, enabling sensitive and specific detection of disease sites with high spatial resolution.

Additionally, nanoscale imaging agents can be designed to enable multimodal imaging, combining multiple imaging modalities within a single nanoparticle platform. For example, nanoparticles incorporating both fluorescent and magnetic components enable simultaneous fluorescence and MRI imaging, offering complementary information about disease localization and progression [62–64].

The development of nanoscale imaging agents holds promise for a wide range of clinical applications, including early cancer detection, cardiovascular disease diagnosis, and neuroimaging. By enabling high-resolution imaging of biological structures and processes, these agents provide clinicians with valuable insights into disease pathology, facilitating early detection, accurate diagnosis, and personalized treatment planning.

3.4 Biomimetic and Responsive Nanomaterials in Medicine

Biomimetic and responsive nanomaterials represent a burgeoning field of research aimed at developing nanoscale platforms that mimic the structure and function of biological systems or respond dynamically to external stimuli. These nanomaterials offer unique advantages for medical applications, including enhanced biocompatibility, targeted drug delivery, and controlled release kinetics.

Biomimetic nanomaterials are designed to replicate the structural and functional properties of biological molecules or systems, enabling them to interact seamlessly with biological tissues and cells [65]. Examples include liposomes, which mimic the structure of cell membranes and can be loaded with therapeutic agents for targeted drug delivery, and protein-based nanoparticles, which leverage the specificity and biocompatibility of natural proteins for drug delivery and imaging applications.

Responsive nanomaterials, on the other hand, are designed to undergo reversible changes in response to specific external stimuli, such as pH, temperature, light, or enzymatic activity [66, 67]. These stimuli-responsive properties enable precise control over drug release kinetics, allowing for on-demand drug delivery at the target site while minimizing off-target effects and systemic toxicity [68–70].

For example, pH-responsive nanoparticles can selectively release their payload in acidic environments characteristic of tumor microenvironments, enabling targeted drug delivery to cancer cells while sparing healthy tissues [71, 72]. Similarly, temperature-responsive nanoparticles can be engineered to release drugs upon exposure to mild hyperthermia, which is often employed as a therapeutic strategy for cancer treatment [73].

Biomimetic and responsive nanomaterials hold promise for applications beyond drug delivery, including tissue engineering, regenerative medicine, and biosensing. Nanomaterials that mimic the extracellular matrix or cellular microenvironments can facilitate cell adhesion, proliferation, and differentiation, offering potential solutions for tissue repair and regeneration.

Responsive nanomaterials can be harnessed for biosensing applications, enabling real-time monitoring of biological analytes or disease biomarkers. By integrating sensing elements within responsive nanomaterials, researchers can develop biosensors capable of detecting specific molecules or pathogens with high sensitivity and specificity, offering diagnostic tools for disease detection and monitoring [74].

Biomimetic and responsive nanomaterials represent a versatile platform for a wide range of medical applications, including targeted drug delivery, tissue engineering, regenerative medicine, and biosensing. By harnessing the unique properties of nanomaterials and leveraging insights from biology and materials science, researchers continue to innovate and advance the field, paving the way for transformative medical technologies with the potential to improve patient outcomes and quality of life. References will be provided where necessary to support the information presented.

4 Clinical Trial Landscape in Nanomedicine

4.1 Regulatory Frameworks for Nanomedicine Clinical Trials

Regulatory oversight plays a critical role in ensuring the safety, efficacy, and quality of nanomedicine products through all stages of development, including clinical trials. Regulatory agencies worldwide have established guidelines and frameworks specific to nanomedicine to address the unique characteristics and challenges associated with these advanced technologies.

In the United States, the Food and Drug Administration (FDA) provides regulatory oversight for nanomedicine products through its Center for Drug Evaluation and Research (CDER) and Center for Devices and Radiological Health (CDRH) [66, 75, 76]. The FDA employs a risk-based approach to evaluate nanomedicine products, considering factors such as nanoparticle size, surface properties, and intended use.

For drug products, nanomedicine developers are required to submit Investigational New Drug (IND) applications to the FDA, outlining preclinical data, manufacturing processes, and proposed clinical trial protocols. The FDA evaluates these applications to ensure the safety of human participants and the scientific validity of the proposed trials. Additionally, the FDA provides guidance documents specific to nanomedicine development, addressing issues such as characterization, toxicology, and clinical trial design.

In Europe, the European Medicines Agency (EMA) oversees the regulation of nanomedicine products through its Committee for Medicinal Products for Human Use (CHMP). Similar to the FDA, the EMA requires developers to submit Marketing Authorization Applications (MAAs) or Clinical Trial Applications (CTAs) for evaluation [77, 78]. The EMA also provides specific guidance on the characterization and assessment of nanomedicine products, emphasizing the importance of understanding the physicochemical properties and biological behavior of nanoparticles.

In addition to regulatory agencies, international organizations such as the International Organization for Standardization (ISO) and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) have developed standards and guidelines for nanomedicine development and evaluation [79]. These standards aim to harmonize regulatory requirements and facilitate global acceptance of nanomedicine products.

Overall, regulatory frameworks for nanomedicine clinical trials are evolving to address the unique challenges posed by these advanced technologies. By providing clear guidance and oversight, regulatory agencies ensure the safety and efficacy of nanomedicine products while fostering innovation and advancing patient care.

4.2 Challenges and Opportunities in Conducting Nanomedicine Trials

While nanomedicine holds tremendous promise for revolutionizing healthcare, conducting clinical trials presents unique challenges that must be addressed to realize this potential fully. These challenges span various aspects of trial design, execution, and interpretation, requiring innovative solutions and collaborative efforts from stakeholders across academia, industry, and regulatory agencies.

One of the primary challenges in nanomedicine trials is the complexity of nanoparticle formulations and their interactions with biological systems. Nanoparticles exhibit unique pharmacokinetic and biodistribution profiles, which may differ significantly from traditional small-molecule drugs [80, 81]. Consequently, optimizing dosing regimens, characterizing nanoparticle behavior *in vivo*, and assessing long-term safety represent significant challenges for clinical trial design.

The heterogeneity of nanoparticle preparations and manufacturing processes poses challenges for standardization and reproducibility. Small variations in nanoparticle size, shape, surface charge, and composition can have profound effects on their biological behavior and therapeutic efficacy. Thus, establishing robust manufacturing processes and quality control measures is essential to ensure consistency and reliability across clinical trials [82].

Another challenge in nanomedicine trials is the development of sensitive and reliable methods for nanoparticle characterization and detection in biological matrices. Conventional analytical techniques may lack the sensitivity or specificity required to accurately quantify nanoparticles in complex biological samples, leading to challenges in assessing pharmacokinetics, biodistribution, and tissue targeting [83].

Furthermore, regulatory agencies face challenges in adapting existing guidelines and frameworks to accommodate the unique properties and behaviours of nanomedicine products. Traditional endpoints and evaluation criteria may need to be revised or supplemented to capture the full spectrum of nanoparticle-mediated effects, such as enhanced permeability and retention (EPR) effects, immune modulation, and off-target effects [83].

Despite these challenges, nanomedicine trials also present opportunities for innovation and advancement in clinical research. Nanoparticle-based therapeutics offer novel mechanisms of action, targeted delivery strategies, and synergistic combinations with conventional therapies, enhancing therapeutic efficacy and reducing adverse effects.

Nanomedicine trials provide opportunities for the development of companion diagnostics and personalized medicine approaches. By integrating imaging agents or biomarkers into nanoparticle formulations, clinicians can tailor treatment regimens to individual patient characteristics, optimizing therapeutic outcomes and improving patient care [84, 85].

Overall, addressing the challenges and harnessing the opportunities presented by nanomedicine trials requires collaborative efforts from researchers, clinicians, regulators, and industry stakeholders. By fostering innovation, standardization, and regulatory harmonization, nanomedicine trials can unlock the full potential of these advanced technologies and improve patient outcomes across a wide range of medical conditions.

4.3 Case Studies Highlighting Successful Clinical Trials in Nanomedicine

Case studies of successful clinical trials in nanomedicine demonstrate the transformative potential of these advanced technologies in addressing unmet medical needs and improving patient outcomes. These trials span various therapeutic areas, including oncology, infectious diseases, neurological disorders, and regenerative medicine, highlighting the versatility and efficacy of nanomedicine approaches.

One notable example is the development of liposomal formulations for the treatment of cancer. Liposomal nanoparticles encapsulate chemotherapeutic drugs, such as doxorubicin or paclitaxel, within lipid bilayers, enabling targeted delivery to tumor tissues while minimizing systemic toxicity [86, 87]. Clinical trials have demonstrated the efficacy of liposomal formulations in improving tumor response and patient survival rates compared to conventional chemotherapy. For instance, the FDA-approved liposomal doxorubicin formulation, Doxil®, has shown promising results in the treatment of ovarian cancer, breast cancer, and Kaposi's sarcoma, demonstrating improved efficacy and reduced cardiotoxicity compared to free doxorubicin [88, 89].

Another example is the use of nanoparticle-based vaccines for infectious diseases. Nanoparticle vaccines, such as virus-like particles (VLPs) or lipid nanoparticles (LNPs), mimic the structure of pathogens and elicit robust immune responses without causing disease. Clinical trials of nanoparticle vaccines have demonstrated their efficacy in preventing infections caused by influenza, human papillomavirus (HPV), and hepatitis B virus (HBV), offering safe and effective alternatives to traditional vaccine approaches [88, 89].

In the field of neurological disorders, nanomedicine approaches hold promise for targeted drug delivery across the blood-brain barrier (BBB). Nanoparticles functionalized with targeting ligands, such as transferrin or antibodies, can traverse the BBB and deliver therapeutics directly to the brain, enabling precise treatment of conditions such as brain tumors [90–92], Alzheimer's disease, and Parkinson's disease. Clinical trials of nanoparticle-based therapies for neurological disorders are ongoing and hold potential for significant therapeutic advancements in the near future [93, 94].

Furthermore, nanomedicine has revolutionized regenerative medicine through the development of scaffold-based tissue engineering strategies. Nanomaterials, such as hydrogels, nanofibers, and nanoparticles, provide scaffolds for cell growth, proliferation, and differentiation, enabling the regeneration of damaged tissues and organs [95–97]. Clinical trials of nanomaterial-based scaffolds for tissue repair and regeneration have shown promising results in applications such as bone regeneration, wound healing, and cartilage repair.

Case studies of successful clinical trials in nanomedicine underscore the transformative impact of these technologies on healthcare delivery. By harnessing the unique properties of nanoparticles, researchers have developed innovative therapies and diagnostics that offer improved efficacy, reduced side effects, and personalized treatment options for patients worldwide. These successes highlight the immense potential of nanomedicine to address unmet medical needs and advance the field of medicine into a new era of precision and effectiveness. References will be provided where necessary to support the information presented.

5 Latest Developments in Nanomedicine Clinical Trials

5.1 Overview of Recent Advancements in Nanomedicine Research

Recent years have witnessed significant advancements in nanomedicine research, propelled by innovations in nanotechnology, biomaterials, and biomedical engineering. These advancements have led to the development of novel nanomedicine platforms with enhanced therapeutic efficacy, improved targeting capabilities, and reduced systemic toxicity.

One notable advancement is the emergence of next-generation nanocarriers for drug delivery, such as polymeric nanoparticles, lipid-based nanoparticles, and inorganic nanoparticles. These nanocarriers offer precise control over drug release kinetics, enabling sustained and controlled drug delivery to the target site [98–100]. Moreover, advancements in surface modification techniques have facilitated the functionalization of nanoparticles with targeting ligands, antibodies, or peptides, enhancing their specificity and affinity for diseased tissues.

Along with medication administration, nanomedicine research has expanded into the realm of theranostics, which integrates diagnostic and therapeutic functionalities within a single nanoplatform. Theranostic nanoparticles enable real-time monitoring of treatment response and disease progression while simultaneously delivering therapeutic agents to the target site [101, 102]. This paradigm shift towards personalized medicine holds promise for improving patient outcomes and optimizing treatment regimens.

Besides, advancements in nanotechnology have led to the development of innovative imaging agents for biomedical imaging modalities, such as magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), and optical imaging [101, 102]. These nanoscale imaging agents offer enhanced sensitivity, resolution, and multiplexing capabilities compared to conventional contrast agents, enabling early detection, accurate diagnosis, and precise localization of disease sites.

In the end, recent advancements in nanomedicine research have expanded the horizons of healthcare delivery, offering new avenues for the diagnosis, treatment, and monitoring of a wide range of medical conditions. By harnessing the power of nanotechnology, researchers continue to push the boundaries of medical science, driving innovation and transforming the landscape of clinical medicine.

5.2 Breakthroughs in Targeted Therapies and Personalized Medicine

Breakthroughs in nanomedicine have revolutionized targeted therapies and personalized medicine, offering tailored treatment options based on individual patient characteristics and disease profiles. Nanoparticle-based therapies enable precise delivery of therapeutics to diseased tissues or cells, minimizing off-target effects and maximizing therapeutic efficacy.

One significant breakthrough is the development of targeted nanomedicines for cancer therapy. By functionalizing nanoparticles with targeting ligands, such as antibodies or peptides, researchers can selectively deliver anticancer drugs to tumor cells while sparing healthy tissues. This targeted approach reduces systemic toxicity and improves patient outcomes by enhancing drug accumulation at the tumor site and minimizing exposure to healthy organs [103–105].

Beyond that, nanomedicine has facilitated the development of personalized treatment regimens through the integration of companion diagnostics and predictive biomarkers. By incorporating imaging agents or biosensors into nanoparticle formulations, clinicians can tailor treatment strategies to individual patient responses, optimizing therapeutic outcomes and minimizing adverse effects.

Another breakthrough is the advent of immunotherapy-based nanomedicines for cancer treatment. Nanoparticle-based immunotherapies, such as immune checkpoint inhibitors and cancer vaccines, harness the body's immune system to recognize and eliminate tumor cells. By encapsulating immunomodulatory agents within nanoparticles, researchers can enhance their stability, bioavailability, and targeting specificity, leading to improved therapeutic responses and prolonged survival in cancer patients.

Meanwhile, nanotechnology has enabled the development of targeted therapies for other medical conditions, such as infectious diseases, inflammatory disorders, and neurodegenerative diseases. Nanoparticle-based therapeutics offer precise control over drug release kinetics and tissue targeting, enhancing therapeutic efficacy and reducing side effects compared to conventional therapies.

Breakthroughs in targeted therapies and personalized medicine have transformed the treatment landscape for a wide range of medical conditions, offering new hope for patients and clinicians alike. By leveraging the unique properties of nanoparticles, researchers continue to innovate and advance the field of nanomedicine, paving the way for more effective and personalized treatment strategies.

5.3 Emerging Trends in Nanoparticle-Based Diagnostics

Advancements in nanomedicine are changing the face of diagnostic imaging by opening up new avenues for precise tracking of disease progression, early detection, and accurate diagnosis. Nanoparticle-based imaging agents provide enhanced sensitivity, resolution, and multiplexing capabilities compared to traditional contrast agents, enabling non-invasive visualization of biological structures and processes at the molecular level.

One emerging trend is the development of multimodal imaging agents that combine multiple imaging modalities within a single nanoparticle platform. These multimodal agents enable complementary information about disease localization, metabolism, and molecular signatures, improving diagnostic accuracy and treatment planning [106, 107]. For example, nanoparticles incorporating both magnetic resonance imaging (MRI) and fluorescence imaging components offer simultaneous anatomical and functional information, facilitating comprehensive assessment of disease status.

Nanoparticle-based imaging agents are being tailored to specific disease biomarkers or molecular targets, enabling targeted imaging of diseased tissues or cells. By conjugating targeting ligands or antibodies onto nanoparticle surfaces, researchers can achieve selective accumulation of imaging agents at the target site, enhancing imaging sensitivity and specificity [106, 107]. This targeted approach holds promise for early detection and precise localization of cancer, cardiovascular disease, and neurological disorders.

Advancements in nanotechnology have led to the development of theranostic nanoparticles that integrate diagnostic and therapeutic functionalities within a single nanoplatform. Theranostic nanoparticles enable real-time monitoring of treatment response and disease progression while simultaneously delivering therapeutic agents to the target site, offering personalized treatment options and improving patient outcomes [108, 109].

Recent developments in nanoparticle-based diagnostics are revolutionizing medical imaging, offering new tools for early disease detection, accurate diagnosis, and personalized treatment planning. By harnessing the unique properties of nanoparticles, researchers continue to push the boundaries of diagnostic imaging, paving the way for more precise and effective healthcare delivery.

5.4 Exploration of Nanotechnology in Gene and Cell Therapies

Nanotechnology holds immense promise for advancing gene and cell therapies, offering novel strategies for the delivery of nucleic acids, proteins, and cells to target tissues or cells. Nanoparticle-based delivery systems provide protection for fragile therapeutic payloads, facilitate cellular uptake and intracellular trafficking, and enable controlled release of therapeutics, enhancing their therapeutic efficacy and safety.

One area of exploration is the development of nanoparticle-based gene delivery systems for the treatment of genetic disorders, cancer, and infectious diseases. Nanoparticles can encapsulate or complex with nucleic acids, such as plasmid DNA, messenger RNA (mRNA), or small interfering RNA (siRNA), and deliver them to target cells or tissues, enabling modulation of gene expression, gene editing, or gene silencing. By engineering nanoparticles with optimal physicochemical properties and surface modifications, researchers can achieve efficient gene delivery with minimal cytotoxicity and off-target effects [110–112].

The advancement of exosome-based medicines for use in cell therapy is another benefit of nanotechnology. Exosomes are extracellular vesicles secreted by cells that play critical roles in intercellular communication and tissue homeostasis. By engineering exosomes with therapeutic cargo, such as nucleic acids, proteins, or drugs, researchers can harness their natural targeting and delivery capabilities to achieve precise modulation of cellular functions and tissue regeneration [113–116]. This approach holds promise for the treatment of various diseases, including neurodegenerative disorders, cardiovascular diseases, and autoimmune diseases.

Nanotechnology has facilitated the development of nanoparticle-based vaccines for infectious diseases and cancer immunotherapy. Nanoparticle vaccines mimic the size, shape, and surface properties of pathogens, eliciting robust immune responses without causing disease. By incorporating nanoparticles with immunostimulatory adjuvants and antigenic proteins or peptides, researchers can enhance vaccine efficacy and induce long-lasting immune memory, offering protection against infectious diseases and malignancies [117, 118].

Nanotechnology has also paved the way for cell-targeted medicines to be designed for use in regenerative medicine. Nanoparticles functionalized with targeting ligands or antibodies can selectively bind to cell surface receptors or markers, facilitating targeted delivery of therapeutic cargo to specific cell populations [119, 120]. This targeted approach enhances the efficiency of cell therapy, promoting tissue repair, regeneration, and functional recovery in various disease conditions, such as neurodegenerative disorders, cardiovascular diseases, and musculoskeletal injuries.

All things considered, the exploration of nanotechnology in gene and cell therapies represents a frontier of biomedical research, offering innovative solutions for the treatment of genetic disorders, cancer, infectious diseases, and regenerative medicine. By leveraging the unique properties of nanoparticles, researchers continue to advance the field of precision medicine, paving the way for personalized and effective therapeutic interventions.

6 Critical Assessment of Nanomedicine Ventures

6.1 Evaluation of the Translational Potential of Nanomedicine Innovations

The translational potential of nanomedicine innovations lies at the heart of their journey from bench to bedside, determining their impact on clinical practice and patient care. Translational research aims to bridge the gap between basic science discoveries and clinical applications, facilitating the development of new diagnostic tools, therapeutic interventions, and medical devices that address unmet medical needs and improve healthcare outcomes.

One key aspect of evaluating the translational potential of nanomedicine innovations is assessing their feasibility for clinical translation. This involves considering factors such as safety, efficacy, scalability, manufacturability, and regulatory approval pathways. Nanomedicine developers must demonstrate the safety and efficacy of their products through rigorous preclinical studies and clinical trials, adhering to regulatory guidelines and quality standards to obtain market approval and ensure patient safety.

Along with this, the translational potential of nanomedicine innovations depends on their ability to address unmet medical needs and provide tangible benefits over existing treatments or diagnostic modalities. Nanomedicine products should offer improved therapeutic outcomes, reduced side effects, enhanced patient compliance, or cost-effective solutions to justify their adoption in clinical practice. Thus, critical evaluation of nanomedicine innovations requires comprehensive assessment of their clinical relevance, market potential, and value proposition compared to standard of care or alternative approaches.

Meanwhile, successful translation of nanomedicine innovations depends on effective collaboration and partnerships across academia, industry, healthcare providers, and regulatory agencies. Collaborative networks and translational research platforms facilitate knowledge exchange, resource sharing, and multidisciplinary expertise, accelerating the development and commercialization of nanomedicine products. By fostering collaborative innovation ecosystems, stakeholders can overcome barriers to translation and maximize the impact of nanomedicine on patient care and public health [84, 121–123].

All things considered, evaluating the translational potential of nanomedicine innovations requires careful consideration of safety, efficacy, clinical relevance, market potential, and collaborative partnerships. By addressing these critical factors, researchers and stakeholders can advance the translation of nanomedicine from bench to bedside, unlocking its full potential to transform healthcare delivery and improve patient outcomes.

6.2 Addressing Safety Concerns and Ethical Considerations

The rapid advancement of nanomedicine technologies has raised important safety concerns and ethical considerations that must be addressed to ensure responsible innovation and ethical practice. Nanoparticles possess unique physicochemical properties that may interact with biological systems in unpredictable ways, leading to potential risks such as toxicity, immunogenicity, and off-target effects [124, 125]. Therefore, comprehensive evaluation of the safety profile of nanomedicine products is essential to mitigate potential risks and ensure patient safety.

One key challenge in nanomedicine safety assessment is predicting and characterizing the biological fate and behavior of nanoparticles in vivo. Nanoparticles undergo complex interactions with biological molecules, cells, and tissues, influencing their pharmacokinetics, biodistribution, and biocompatibility [124, 125]. Researchers employ a variety of experimental techniques, including in vitro assays, animal studies, and computational modeling, to assess the safety profile of nanoparticles and identify potential hazards or adverse effects.

Ethical considerations arise in the development and application of nanomedicine technologies, particularly concerning issues such as patient consent, privacy, equity, and access to healthcare [126]. Researchers and clinicians must adhere to ethical principles and regulatory guidelines governing human subjects research, ensuring informed consent, protection of privacy, and equitable access to experimental therapies. Additionally, considerations of social justice, equity, and distributive justice are essential to address disparities in healthcare access and ensure that nanomedicine benefits reach underserved populations.

The responsible translation and commercialization of nanomedicine products require transparent communication, public engagement, and stakeholder involvement to build trust, foster accountability, and address societal concerns [127, 128]. By promoting open dialogue and inclusive decision-making processes, stakeholders can navigate ethical challenges, build consensus, and promote responsible innovation in nanomedicine.

Addressing safety concerns and ethical considerations is paramount to the responsible development and application of nanomedicine technologies. By prioritizing patient safety, adhering to ethical principles, and engaging stakeholders in transparent and inclusive decision-making processes, researchers and policymakers can foster trust, promote social acceptance, and maximize the benefits of nanomedicine for healthcare and society.

6.3 Impact of Nanomedicine on Healthcare Systems and Patient Outcomes

The impact of nanomedicine on healthcare systems and patient outcomes is multifaceted, encompassing improvements in disease management, therapeutic efficacy, healthcare delivery, and resource utilization. Nanomedicine technologies offer potential solutions to pressing healthcare challenges, such as personalized medicine, targeted therapy, early detection, and precision diagnostics, which have the potential to transform healthcare delivery and improve patient outcomes.

One significant impact of nanomedicine is its ability to enable personalized medicine approaches tailored to individual patient characteristics, disease profiles, and treatment responses [129–131]. Nanoparticle-based diagnostics offer sensitive and specific detection of disease biomarkers, enabling early diagnosis, accurate prognosis, and personalized treatment planning. Similarly, targeted nanomedicine therapies deliver therapeutics directly to diseased tissues or cells, maximizing efficacy while minimizing systemic toxicity, adverse effects, and treatment-related complications [132, 133].

Nanomedicine has the potential to revolutionize drug discovery and development processes, accelerating the translation of basic science discoveries into clinical applications. Nanotechnology platforms enable high-throughput screening, combinatorial chemistry, and rational design of therapeutic agents, facilitating the identification of novel drug candidates, optimization of drug delivery systems, and enhancement of drug efficacy and safety profiles. By streamlining drug development pipelines and reducing development costs and timelines, nanomedicine technologies contribute to the efficiency and productivity of pharmaceutical research and development.

Nanomedicine innovations have implications for healthcare delivery models, healthcare economics, and healthcare access. Nanotechnology-based diagnostics and therapeutics offer opportunities for decentralized healthcare delivery, point-of-care testing, and telemedicine applications, extending the reach of healthcare services to underserved populations, remote regions, and resource-limited settings. Additionally, nanomedicine technologies have the potential to reduce healthcare costs by improving diagnostic accuracy, reducing hospitalizations and treatment durations, and optimizing resource allocation through targeted and personalized interventions [134].

The impact of nanomedicine on healthcare systems and patient outcomes is profound and far-reaching, offering transformative solutions to healthcare challenges and improving the quality, accessibility, and affordability of healthcare services. By harnessing the full potential of nanomedicine technologies and addressing associated challenges, stakeholders can realize the promise of precision medicine and personalized healthcare, advancing the well-being and health outcomes of individuals and communities.

7 Future Prospects and Challenges

7.1 Predictions for the Future of Nanomedicine Ventures

The future of nanomedicine ventures holds immense promise for revolutionizing healthcare delivery and advancing medical science. As technology continues to evolve and our understanding of nanomaterials and biological systems deepens, we can anticipate several key trends and developments that will shape the landscape of nanomedicine in the coming years.

One prediction is the continued expansion of personalized medicine approaches enabled by nanomedicine technologies. Advances in genomics, proteomics, and biomarker discovery will facilitate the identification of disease signatures and therapeutic targets, guiding the development of tailored nanomedicine therapies and diagnostics [135, 136]. Nanoparticle-based drug delivery systems will become increasingly sophisticated, offering precise control over drug release kinetics, tissue targeting, and therapeutic payloads, optimizing treatment outcomes and minimizing adverse effects.

The integration of artificial intelligence (AI) and machine learning algorithms into nanomedicine research and clinical practice will enable data-driven decision-making, predictive modeling, and personalized treatment algorithms. AI-powered platforms will analyze large datasets of patient demographics, clinical parameters, and molecular profiles to identify patterns, predict treatment responses, and optimize therapeutic regimens, leading to more effective and efficient healthcare delivery [135, 136].

Furthermore, the convergence of nanotechnology with other cutting-edge technologies, such as 3D printing, microfluidics, and wearable sensors, will open new frontiers in personalized medicine and point-of-care diagnostics [137]. Miniaturized, portable nanodevices will enable real-time monitoring of physiological parameters, biomarker levels, and treatment responses, empowering patients to actively participate in their healthcare management and facilitating early intervention and preventive care strategies [137].

The rise of collaborative innovation ecosystems and global partnerships will drive the translation of nanomedicine discoveries from bench to bedside, fostering interdisciplinary collaborations among researchers, clinicians, industry partners, and regulatory agencies. Open-access platforms, shared research infrastructures, and collaborative funding initiatives will facilitate knowledge exchange, technology transfer, and regulatory harmonization, accelerating the development and commercialization of nanomedicine products [137].

In a nutshell, the future of nanomedicine ventures is bright, with unprecedented opportunities for improving patient outcomes, enhancing healthcare access, and addressing unmet medical needs. By embracing innovation, collaboration, and patient-centric approaches, stakeholders can harness the full potential of nanomedicine to usher in a new era of precision medicine and personalized healthcare.

7.2 Anticipated Advancements in Clinical Trial Methodologies

The field of clinical trial methodologies is poised for significant advancements driven by innovations in nanomedicine research, technology, and regulatory frameworks. As nanomedicine continues to mature as a field, several anticipated advancements in clinical trial methodologies are expected to emerge, facilitating the translation of nanomedicine innovations from preclinical studies to clinical applications.

One anticipated advancement is the development of novel trial designs tailored to the unique characteristics of nanomedicine products. Traditional clinical trial designs may not be well-suited to evaluate nanomedicine therapies, which often exhibit complex pharmacokinetics, tissue distribution, and therapeutic mechanisms. Adaptive trial designs, Bayesian statistics, and platform trials will enable flexible, dynamic approaches to data analysis, sample size adjustments, and treatment allocation, optimizing trial efficiency and statistical power [138–140].

The incorporation of innovative biomarkers and surrogate endpoints into clinical trial protocols will enhance the efficiency and sensitivity of outcome assessments for nanomedicine therapies. Biomarkers of treatment response, disease progression, and patient stratification will enable early identification of therapeutic efficacy, patient selection, and treatment optimization, guiding clinical decision-making and regulatory approval processes [141, 142].

Advancements in imaging technologies and molecular diagnostics will revolutionize clinical trial methodologies by enabling non-invasive monitoring of treatment responses, disease progression, and pharmacokinetics. Multimodal imaging techniques, such as positron emission tomography (PET)/magnetic resonance imaging (MRI) and optical coherence tomography (OCT), will provide complementary information about disease biology, treatment effects, and patient outcomes, facilitating objective and quantitative assessments of therapeutic efficacy [141, 142].

The adoption of patient-centered trial designs and digital health technologies will enhance patient engagement, adherence, and retention in clinical trials. Mobile health (mHealth) apps, wearable devices, and remote monitoring platforms will enable real-time collection of patient-reported outcomes, adherence data, and physiological parameters, enhancing data quality, trial efficiency, and participant satisfaction.

Anticipated advancements in clinical trial methodologies will revolutionize the evaluation of nanomedicine therapies, offering innovative trial designs, biomarkers, imaging techniques, and digital health technologies to enhance trial efficiency, sensitivity, and patient-centeredness. By embracing these advancements, stakeholders can accelerate the translation of nanomedicine innovations from the laboratory to the clinic, bringing new treatments and diagnostics to patients in need.

7.3 Addressing Potential Challenges and Mitigating Risks in Nanomedicine Development

Despite the tremendous promise of nanomedicine, several challenges and risks must be addressed to ensure responsible development, regulatory approval, and safe clinical translation of nanomedicine products. These challenges span scientific, regulatory, ethical, and commercial domains and require collaborative efforts from stakeholders across academia, industry, government, and civil society.

One significant challenge is the potential for nanomaterial toxicity and immunogenicity, which may arise from the physicochemical properties, surface characteristics, and biodistribution of nanoparticles [141, 142]. Comprehensive preclinical safety assessment strategies, including in vitro assays, animal studies, and computational modelling, are essential to evaluate the biocompatibility, pharmacokinetics, and long-term effects of nanomedicine products and identify potential hazards or adverse effects.

Regulatory agencies face challenges in establishing standardized testing protocols, safety guidelines, and approval pathways for nanomedicine products due to their unique properties and mechanisms of action. Harmonization of regulatory frameworks, international collaboration, and stakeholder engagement are critical to ensure consistent, science-based regulation of nanomedicine across different jurisdictions and facilitate global market access for innovative nanomedicine therapies and diagnostics [141, 142].

Ethical considerations surrounding nanomedicine research, clinical trials, and commercialization must be carefully addressed to uphold patient rights, privacy, autonomy, and informed consent. Transparent communication, public engagement, and ethical oversight are essential to build trust, foster accountability, and address societal concerns about nanomedicine safety, efficacy, and equitable access [127, 128].

Commercialization challenges, such as intellectual property rights, market competition, and reimbursement strategies, may hinder the translation of nanomedicine innovations from research laboratories to commercial products [143, 144]. Collaborative innovation ecosystems, technology transfer initiatives, and public-private partnerships can facilitate the transition from academic research to commercialization, providing support for startups, small-to-medium enterprises (SMEs), and innovators to navigate the complexities of the nanomedicine market.

Addressing potential challenges and mitigating risks in nanomedicine development requires concerted efforts from researchers, regulators, policymakers, industry partners, and patient advocates. By proactively identifying and addressing safety concerns, regulatory hurdles, ethical considerations, and commercialization challenges, stakeholders can foster responsible innovation, ensure patient safety, and maximize the societal benefits of nanomedicine.

8 Conclusion

8.1 Summarizing Key Findings

In reflection upon the extensive exploration of nanomedicine ventures, several key findings emerge, illuminating the transformative potential of nanotechnology in healthcare innovation and patient care. These insights underscore the collaborative efforts, scientific advancements, and ethical considerations inherent in the journey of nanomedicine ventures.

Firstly, the significance of interdisciplinary collaboration emerges as a linchpin in driving progress within nanomedicine ventures. By harnessing diverse expertise from fields such as chemistry, biology, engineering, and medicine, interdisciplinary teams can synergize knowledge and perspectives to develop innovative nanomedicine solutions tailored to specific disease targets and patient needs.

Secondly, the pivotal role of clinical trials in validating nanomedicine applications becomes evident, highlighting the importance of robust evidence generation for regulatory approval and clinical adoption. Clinical trials serve as the crucible where nanomedicine innovations are rigorously evaluated for safety, efficacy, and translational potential, guiding decision-making and shaping clinical practice.

The ethical imperative of addressing safety concerns and ethical considerations emerges as a guiding principle in nanomedicine ventures. Responsible development and deployment of nanotechnology-based interventions necessitate proactive risk assessment, ethical governance, and stakeholder engagement to ensure patient safety, privacy, and societal trust.

The transformative impact of nanomedicine on healthcare delivery and patient outcomes becomes apparent, with nanotechnology poised to revolutionize disease diagnosis, treatment, and monitoring. From targeted drug delivery systems to personalized diagnostics and regenerative therapies, nanomedicine offers innovative solutions to unmet medical needs, improving patient quality of life and healthcare outcomes.

The journey through nanomedicine ventures reveals a tapestry of collaborative innovation, scientific discovery, and ethical stewardship. By embracing interdisciplinary collaboration, rigorous evidence-based practice, and ethical principles, nanomedicine ventures hold the promise of transforming healthcare delivery and shaping the future of medicine. Through continued collaboration, innovation, and ethical leadership, nanomedicine ventures can realize their full potential in advancing patient care and societal well-being.

8.2 Emphasizing the Role of Nanomedicine in Shaping the Future of Healthcare

Nanomedicine stands at the forefront of biomedical innovation, offering transformative solutions to pressing healthcare challenges and improving the quality, accessibility, and affordability of healthcare services. By harnessing the unique properties of nanomaterials, researchers and clinicians can develop targeted therapies, personalized diagnostics, and regenerative treatments that offer unprecedented precision and efficacy.

The role of nanomedicine in shaping the future of healthcare extends beyond the development of novel technologies to encompass a paradigm shift towards personalized medicine, preventive care, and patient-centered approaches. Nanotechnology-based interventions empower patients to take control of their health, providing tailored solutions that address individual needs, preferences, and genetic predispositions.

Nanomedicine has the potential to revolutionize healthcare delivery models, enabling decentralized, community-based care, telemedicine, and remote monitoring solutions that extend the reach of healthcare services to underserved populations, remote regions, and resource-limited settings. By leveraging digital health technologies and point-of-care diagnostics, nanomedicine enhances healthcare access and promotes early intervention, leading to better health outcomes and reduced healthcare disparities.

Nanomedicine represents a transformative force in healthcare, offering innovative solutions that redefine the boundaries of medical science and improve the lives of patients worldwide. As we continue to unlock the full potential of nanotechnology, collaboration, innovation, and ethical stewardship will be essential in realizing the promise of nanomedicine and ushering in a new era of precision medicine and personalized healthcare.

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Theranostics: Integrated Diagnostics and Therapy Using Nanomedicine

Varsha Singh

1 Introduction to Theranostics and Nanomedicine

Theranostics represents a pivotal convergence of diagnostic and therapeutic strategies within healthcare. At its essence, theranostics embodies a proactive approach to patient care, where the integration of diagnostics and therapy into a single platform enables personalized and precise treatment regimens [1]. By combining diagnostic capabilities with therapeutic interventions, theranostics empowers clinicians to monitor disease progression in real-time and tailor treatment strategies to individual patient needs [2]. This proactive approach enhances therapeutic efficacy and minimizes adverse effects, ultimately improving patient outcomes and quality of life [3]. Nanomedicine, leveraging the principles of nanotechnology, has emerged as a transformative field in healthcare, offering novel solutions for diagnosing and treating diseases at the nanoscale [4]. Nanoparticles, characterized by their unique physicochemical properties, serve as versatile drug delivery, imaging, and diagnostics platforms. Engineered at the nanoscale, these particles exhibit enhanced biocompatibility, prolonged circulation time, and targeted delivery to specific tissues or cells [5]. Nanomedicine has revolutionized traditional drug delivery approaches by overcoming biological barriers and enabling precise control over drug release kinetics [6]. Moreover, nanotechnology has opened new avenues for non-invasive imaging techniques, allowing for early detection and accurate characterization of diseases.

Integrating diagnostics and therapy through theranostics represents a paradigm shift in disease management, offering several key advantages over conventional approaches. By combining diagnostic imaging modalities with therapeutic interventions, theranostic platforms enable early detection of diseases, precise localization

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of pathological lesions, and real-time monitoring of treatment efficacy [1]. This integrated approach enhances therapeutic outcomes and minimizes adverse effects associated with systemic drug administration, thereby improving patient compliance. Moreover, the ability to tailor treatment regimens based on real-time feedback enhances the efficacy of interventions, ultimately leading to better patient outcomes [4].

The evolution of theranostics within the field of nanomedicine has been characterized by continuous innovation in nanoparticle design, imaging techniques, and therapeutic modalities. Early efforts focused on developing multifunctional nanoparticles capable of simultaneous imaging and drug delivery, laying the foundation for more sophisticated theranostic platforms with enhanced targeting capabilities. Recent advancements, such as stimuli-responsive nanoparticles and theranostic nanocomposites, have further expanded the scope of theranostic applications, paving the way for personalized medicine and precision therapeutics [7]. These advancements underscore the transformative potential of theranostics in improving patient care and advancing the field of medicine.

2 Principles of Theranostic Nanomedicine

2.1 Nanoparticle Design and Synthesis

Nanoparticle design and synthesis are fundamental aspects of theranostic nanomedicine, influencing the performance and efficacy of theranostic platforms. The theranostic nanoparticles' design involves carefully considering various factors, including size, shape, surface chemistry, and functionalization [8]. Size plays a crucial role in determining nanoparticle biodistribution and cellular uptake, with smaller particles exhibiting enhanced tumor penetration and cellular internalization [9]. Additionally, the shape of nanoparticles can influence their interactions with biological systems, affecting circulation time and targeting efficiency [10]. Surface chemistry and functionalization strategies enable the attachment of targeting ligands, imaging agents, and therapeutic payloads to nanoparticles, facilitating their multifunctionality [11]. Various synthesis methods, such as chemical synthesis, self-assembly, and bottom-up fabrication, offer precise control over nanoparticle properties, allowing for the development of theranostic platforms tailored to specific applications [12].

2.2 Multifunctionality for Simultaneous Diagnostics and Therapy

The multifunctionality of theranostic nanoparticles lies at the heart of their utility in simultaneous diagnostics and therapy. By incorporating diagnostic and therapeutic functionalities into a single platform, theranostic nanoparticles enable real-time monitoring of disease progression and targeted delivery of therapeutic agents [13]. Diagnostic functionalities, such as imaging agents or biosensors, allow for non-invasive visualization and characterization of diseased tissues, providing valuable information for treatment planning [14]. Meanwhile, therapeutic functionalities, including drug encapsulation or gene delivery, enable precise and localized treatment of diseases, minimizing systemic side effects. The synergistic combination of diagnostic and therapeutic capabilities within theranostic nanoparticles offers significant advantages over conventional approaches, enhancing treatment efficacy and patient outcomes [11].

2.3 Targeting Strategies for Enhanced Specificity

Targeting strategies are crucial in enhancing the specificity and efficacy of theranostic nanoparticles. Active targeting involves the conjugation of targeting ligands, such as antibodies or peptides, to the surface of nanoparticles, enabling specific recognition and binding to molecular targets overexpressed on diseased cells [15]. Passive targeting exploits the enhanced permeability and retention (EPR) effect, wherein nanoparticles preferentially accumulate in tumors due to leaky vasculature and impaired lymphatic drainage [16]. Combination strategies that leverage active and passive targeting mechanisms offer synergistic benefits, further enhancing the specificity and efficacy of theranostic nanoparticles [17]. Moreover, the development of stimuli-responsive nanoparticles allows for the triggered release of therapeutic payloads in response to endogenous or exogenous stimuli, further improving targeting efficiency and therapeutic outcomes.

3 Applications of Theranostic Nanomedicine

3.1 Cancer Therapy

Cancer therapy is one of the most prominent applications of theranostic nanomedicine, leveraging the multifunctionality of nanoparticles for targeted drug delivery and non-invasive imaging. Nanoparticle-based drug delivery systems enable the selective accumulation of therapeutic agents in tumor tissues while minimizing off-target effects [18]. Additionally, theranostic nanoparticles equipped with imaging agents, such as quantum dots or magnetic nanoparticles, facilitate the visualization of tumors and metastatic lesions, aiding in treatment planning and monitoring [19]. Moreover, stimuli-responsive nanoparticles offer precise control over drug release kinetics, allowing for on-demand drug release triggered by specific environmental cues within the tumor microenvironment [20]. These advancements in theranostic nanomedicine promise to improve the efficacy and safety of cancer therapy, ultimately leading to better patient outcomes.

3.2 Cardiovascular Disease Management

Theranostic nanomedicine has emerged as a promising approach for the management of cardiovascular diseases, including atherosclerosis and myocardial infarction. Nanoparticle-based imaging agents, such as iron oxide nanoparticles or gold nanorods, enable non-invasive imaging of atherosclerotic plaques and assess plaque vulnerability [21]. Furthermore, theranostic nanoparticles can be functionalized with anti-inflammatory agents or thrombolytic drugs to mitigate plaque inflammation and prevent thrombus formation [22]. Additionally, targeted delivery of therapeutic agents to the site of vascular injury or myocardial infarction enhances treatment efficacy while minimizing systemic side effects [23]. Integrating diagnostic imaging and therapeutic interventions within theranostic nanoparticles offers a holistic approach to cardiovascular disease management, enabling early detection, precise treatment, and monitoring of therapeutic outcomes.

3.3 Neurological Disorders

Theranostic nanomedicine holds promise for the management of neurological disorders, including Alzheimer's disease, Parkinson's disease, and brain tumors. Nanoparticle-based imaging agents, such as fluorescent dyes or magnetic nanoparticles, enable non-invasive imaging of pathological lesions and assessment of disease progression [24]. Moreover, theranostic nanoparticles can be engineered to deliver neuroprotective agents or therapeutic drugs across the blood-brain barrier, facilitating targeted treatment of neurological disorders [25]. Additionally, stimuli-responsive nanoparticles offer the potential for controlled drug release within the brain, minimizing systemic side effects and enhancing treatment efficacy [26]. The development of theranostic nanomedicine for neurological disorders represents a significant advancement in personalized medicine, offering tailored treatment strategies based on individual patient needs.

3.4 Infectious Diseases

Theranostic nanomedicine has emerged as a promising approach for managing infectious diseases, including bacterial, viral, and fungal infections. Nanoparticle-based imaging agents, such as fluorescent nanoparticles or quantum dots, enable rapid and sensitive detection of pathogens in biological samples [27]. Additionally, theranostic nanoparticles can be functionalized with antimicrobial agents or antiviral drugs to target and eradicate microbial pathogens [28]. Furthermore, targeted delivery of therapeutic agents to infected tissues or cells enhances treatment efficacy while minimizing systemic toxicity [29]. Integrating diagnostic imaging and therapeutic interventions within theranostic nanoparticles offers a multifaceted approach to infectious disease management, enabling early detection, precise treatment, and monitoring of therapeutic outcomes.

3.5 Musculoskeletal Disorders

Theranostic nanomedicine holds promise for managing musculoskeletal disorders, including osteoarthritis, rheumatoid arthritis, and bone tumors [30]. Nanoparticle-based imaging agents, such as magnetic resonance imaging (MRI) contrast agents or fluorescent nanoparticles, enable non-invasive imaging of joint inflammation, cartilage degradation, and bone lesions [31]. Moreover, theranostic nanoparticles can be functionalized with anti-inflammatory agents or disease-modifying drugs to mitigate inflammation and promote tissue regeneration. Additionally, targeted delivery of therapeutic agents to affected joints or bone tissues enhances treatment efficacy while minimizing systemic side effects [32]. Integrating diagnostic imaging and therapeutic interventions within theranostic nanoparticles offers a comprehensive approach to musculoskeletal disorder management, enabling early detection, precise treatment, and monitoring of therapeutic outcomes.

4 Diagnostic Modalities in Theranostic Nanomedicine

4.1 Imaging Techniques

Imaging techniques play a central role in theranostic nanomedicine, enabling non-invasive visualization and characterization of diseases. Various imaging modalities, including MRI, computed tomography (CT), positron emission tomography (PET), and optical imaging, offer unique advantages for theranostic applications [33]. MRI is widely used in theranostic nanomedicine due to its high spatial resolution and excellent soft tissue contrast. Superparamagnetic iron oxide nanoparticles (SPIONs) and gadolinium-based contrast agents are commonly employed as MRI contrast agents, enabling sensitive detection of tumors and assessment of treatment response [34]. CT imaging provides detailed anatomical information with high spatial resolution, making it suitable for detecting tumors and assessing vascular abnormalities [35]. Nanoparticle-based contrast agents, such as gold nanoparticles and iodine-based agents, enhance CT imaging sensitivity and enable functional imaging of physiological processes [36]. PET imaging offers a quantitative assessment of biological processes at the molecular level, making it valuable for monitoring disease progression and evaluating treatment efficacy. Radiolabeled nanoparticles, such as 18F-fluorodeoxyglucose (FDG) and 64Cu-labeled nanoparticles, enable sensitive detection of tumors and metastatic lesions [37]. Optical imaging techniques, including fluorescence and bioluminescence, provide real-time visualization of biological processes at the cellular and molecular levels [38]. Fluorescent nanoparticles, such as quantum dots and organic dyes, offer high sensitivity and multiplexing capabilities for imaging various disease markers.

4.2 Biomarker Detection

Biomarker detection is crucial in theranostic nanomedicine, enabling disease diagnosis and personalized treatment. Proteomics and genomics-based approaches offer powerful tools for identifying disease-specific biomarkers and monitoring treatment response. Proteomics involves the comprehensive analysis of proteins expressed in biological samples, offering insights into disease pathogenesis and progression. Nanoparticle-based biosensors, such as surface-enhanced Raman scattering (SERS) and plasmonic nanoparticles, enable sensitive detection of protein biomarkers associated with various diseases, including cancer and cardiovascular disorders [13]. Genomics studies genetic variations and mutations associated with disease susceptibility and progression. Nanoparticle-based gene sequencing technologies, such as nanopore sequencing and DNA-encoded nanoparticles, enable rapid and accurate detection of genetic biomarkers for precision medicine applications [12].

4.3 Real-Time Monitoring of Disease Progression

Real-time monitoring of disease progression is essential for optimizing treatment regimens and assessing therapeutic outcomes in theranostic nanomedicine. Continuous monitoring of disease biomarkers and physiological parameters enables early detection of treatment response and timely intervention. Nanoparticle-based biosensors, such as electrochemical sensors and microfluidic devices, offer real-time monitoring of disease biomarkers in biological fluids, such as blood and urine [39]. These biosensors provide rapid and sensitive detection of biomarkers associated with various diseases, including cancer, infectious diseases, and metabolic disorders.

Advanced imaging techniques, such as dynamic contrast-enhanced MRI and fluorescence imaging, enable real-time visualization of disease progression and treatment response in preclinical and clinical settings. These imaging modalities offer insights into tumor growth kinetics, vascular permeability, and tissue perfusion, facilitating personalized treatment planning and optimization [40].

Diagnostic modalities in theranostic nanomedicine encompass a diverse range of imaging techniques and biomarker detection strategies, enabling non-invasive visualization, early diagnosis, and real-time monitoring of disease progression [41]. The integration of imaging and biomarker detection within theranostic nanoparticles offers unprecedented opportunities for personalized medicine and precision therapeutics.

5 Therapeutic Applications of Nanomedicine

5.1 Drug Delivery Systems

Nanomedicine has revolutionized drug delivery systems, offering targeted and controlled release of therapeutic agents to diseased tissues while minimizing systemic side effects [42]. Nanoparticle-based drug delivery systems encompass diverse materials, including liposomes, polymeric nanoparticles, dendrimers, and inorganic nanoparticles, each with unique properties and applications [43].

Liposomes are lipid-based vesicles encapsulate drugs within their aqueous core or lipid bilayer. Liposomal formulations offer several advantages, including biocompatibility, versatility, and tunable release kinetics [44]. PEGylated liposomes, such as Doxil® and Myocet®, have been approved to treat various cancers, demonstrating enhanced pharmacokinetics and reduced cardiotoxicity compared to conventional chemotherapy [45].

Polymeric nanoparticles are colloidal particles composed of biodegradable polymers, such as poly(lactic-co-glycolic acid) (PLGA) and polyethylene glycol (PEG). These nanoparticles enable the sustained release of drugs and can be surface-functionalized for targeted delivery [46]. Abraxane®, a nanoparticle albumin-bound paclitaxel formulation, has shown improved efficacy and reduced toxicity in treating breast and pancreatic cancer [47].

Dendrimers are highly branched macromolecules with well-defined structures, offering precise control over drug loading and release [48]. Dendrimer-based drug delivery systems have been investigated for treating cancer, infectious diseases, and inflammatory disorders, demonstrating enhanced cellular uptake and therapeutic efficacy [49].

Inorganic nanoparticles, such as gold nanoparticles and magnetic nanoparticles, offer unique properties for drug delivery applications. Gold nanoparticles can be functionalized by targeting ligands and therapeutic agents to treat cancer and inflammatory diseases [50]. Magnetic nanoparticles, on the other hand, enable targeted drug delivery and hyperthermia therapy through external magnetic field manipulation [51].

6 Gene Therapy

Gene therapy holds promise for treating genetic disorders, cancer, and infectious diseases, offering targeted modulation of gene expression and regulation [52]. Nanoparticle-based gene delivery systems facilitate the efficient delivery of nucleic acids, including DNA, RNA, and small interfering RNA (siRNA), to target cells while protecting them from degradation [53].

Lipid nanoparticles are widely used for nucleic acid delivery because they encapsulate and protect them from enzymatic degradation [54]. Lipid-based formulations, such as RNA-lipoplexes and lipid nanoparticles, have shown promise for delivering mRNA vaccines and gene editing tools, including CRISPR/Cas9 [55].

Polymeric nanoparticles offer a versatile platform for gene delivery, allowing for tunable release kinetics and surface functionalization [56]. Polymeric vectors, such as polyethyleneimine (PEI) and poly(beta-amino esters), facilitate efficient gene transfection and have been investigated for treating cancer and genetic disorders [57].

Dendrimers exhibit unique properties for gene delivery applications, including high surface-to-volume ratio and multivalent surface functionality [48]. Dendrimer-based gene delivery systems offer precise control over nucleic acid loading and release, enabling targeted gene silencing and editing [49].

Viral vectors, such as adeno-associated viruses (AAV) and lentiviruses, are commonly used for gene therapy due to their high transduction efficiency and stable gene expression [58]. Viral vectors encapsulated within nanoparticles offer enhanced safety and efficacy profiles, overcoming immunogenicity and cytotoxicity concerns associated with naked viral vectors.

7 Photothermal and Photodynamic Therapy

Photothermal therapy (PTT) and photodynamic therapy (PDT) are emerging strategies for cancer treatment, offering precise and minimally invasive approaches for tumor ablation and eradication [59].

Photothermal therapy utilizes light-absorbing agents, such as gold nanoparticles and carbon nanotubes, to convert near-infrared (NIR) light into heat, inducing localized hyperthermia and tumor cell death [60]. Gold nanorods, functionalized with targeting ligands, have demonstrated selective accumulation in tumors and effective photothermal ablation of cancer cells in preclinical models [61].

Photodynamic therapy involves the administration of photosensitizing agents, such as porphyrins and phthalocyanines, followed by irradiation with light of specific wavelengths to generate reactive oxygen species (ROS) and induce tumor cell apoptosis [62]. Nanoparticle-based photosensitizers, such as liposomal formulations and silica nanoparticles, offer enhanced stability and tumor-targeting capabilities, improving therapeutic efficacy [63]. Combined photothermal and photodynamic therapy (PTT/PDT) approaches leverage the synergistic effects of hyperthermia and

ROS generation for enhanced tumor ablation and eradication [64]. Nanoparticle-based theranostic platforms, capable of simultaneous imaging and phototherapy, offer real-time monitoring of treatment response and optimization of therapeutic regimens [65].

Therapeutic applications of nanomedicine, including drug delivery systems, gene therapy, and photothermal/photodynamic therapy, offer innovative approaches for treating various diseases, ranging from cancer to genetic disorders. Integrating nanotechnology with therapeutic modalities enables targeted and personalized treatment strategies (Fig. 1).

8 Advancements in Theranostic Nanomedicine

8.1 Recent Research and Case Studies

Recent advancements in theranostic nanomedicine have demonstrated the potential of this field in revolutionizing disease diagnosis, treatment, and monitoring. Numerous studies have showcased the efficacy and versatility of theranostic nanoparticles in various biomedical applications, ranging from cancer therapy to infectious disease management.

Recent Research

In a recent study, theranostic nanoparticles were developed for the targeted delivery of chemotherapeutic agents to tumor tissues while simultaneously monitoring treatment response using non-invasive imaging techniques [66]. The nanoparticles, composed of biodegradable polymers and fluorescent dyes, exhibited enhanced tumor accumulation and prolonged circulation time, leading to improved therapeutic outcomes in preclinical models of breast cancer.

Another notable research effort focused on developing theranostic nanoparticles for treating Alzheimer's [67]. The nanoparticles, functionalized with neuroprotective peptides and imaging agents, enabled targeted delivery to amyloid-beta plaques in the brain, facilitating early diagnosis and monitoring of disease progression. This study highlights the potential of theranostic nanomedicine in managing neurodegenerative disorders.

Case Studies

A compelling case demonstrated the clinical translation of theranostic nanoparticles for treating glioblastoma, a deadly form of brain cancer [68]. The nanoparticles, loaded with chemotherapeutic drugs and MRI contrast agents, enabled precise drug

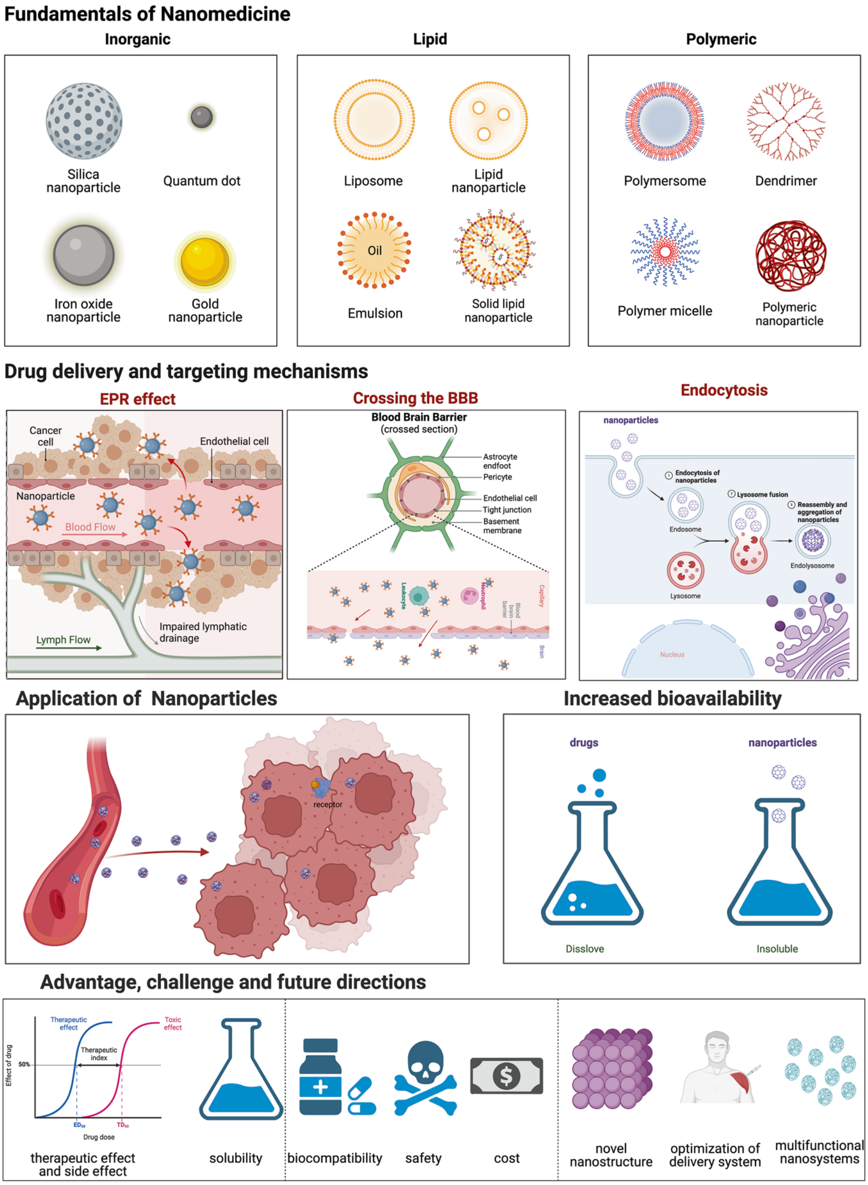
Fundamentals of Nanomedicine
Inorganic
Lipid
Polymeric
Silica nanoparticle
Quantum dot
Iron oxide nanoparticle
Gold nanoparticle
Liposome
Lipid nanoparticle
Oil
Emulsion
Solid lipid nanoparticle
Polymersome
Dendrimer
Polymer micelle
Polymeric nanoparticle
Drug delivery and targeting mechanisms
EPR effect
Cancer cell
Endothelial cell
Nanoparticle
Blood Flow
Impaired lymphatic drainage
Lymph Flow
Crossing the BBB
Blood Brain Barrier
(crossed section)
Astrocyte endfoot
Pericyte
Endothelial cell
Tight junction
Basement membrane
Leukocyte
Neutrophil
Blood brain barrier
Capillary
Brain
Endocytosis
nanoparticles
① Endocytosis of nanoparticles
② Lysosome fusion
③ Reassembly and aggregation of nanoparticles
Endosome
Lysosome
Endolysosome
Nucleus
Application of Nanoparticles
receptor
Increased bioavailability
drugs
nanoparticles
Disslove
Insoluble
Advantage, challenge and future directions
Therapeutic effect
Toxic effect
Therapeutic index
50%
Effect of drug
ED50
TD50
Drug dose
therapeutic effect and side effect
solubility
biocompatibility
safety
cost
novel nanostructure
optimization of delivery system
multifunctional nanosystems

Fig. 1 The figure legend illustrates the fundamental aspects driving the development of nanomedicine. It highlights the pivotal role of nanotechnology in facilitating advanced drug delivery and targeting mechanisms

delivery to tumor tissues while providing real-time imaging guidance during surgery. This integrated approach improved surgical outcomes and prolonged survival in patients with glioblastoma. Similarly, a case report documented the successful application of theranostic nanoparticles in treating drug-resistant bacterial infections [69]. The nanoparticles, functionalized with antimicrobial peptides and fluorescent probes, enabled targeted eradication of bacterial biofilms while enabling real-time monitoring of treatment response using fluorescence imaging. This case highlights the potential of theranostic nanomedicine in combating antibiotic resistance and improving patient outcomes.

8.2 Innovative Approaches to Nanoparticle Engineering

Advancements in nanoparticle engineering have paved the way for developing next-generation theranostic platforms with enhanced functionality and biocompatibility. Innovative nanoparticle design and synthesis approaches have enabled precise control over particle size, shape, surface chemistry, and payload loading capacity.

Multifunctional Nanoparticles

Multifunctional nanoparticles, capable of integrating diagnostic imaging, therapeutic delivery, and targeting functionalities within a single platform, have emerged as promising theranostic agents. These nanoparticles can be engineered to incorporate imaging modalities, such as MRI, fluorescence, positron emission tomography (PET), and therapeutic agents, including chemotherapeutic drugs, nucleic acids, and proteins [70]. By combining multiple functionalities within a single nanoplatform, multifunctional nanoparticles offer synergistic effects for enhanced disease diagnosis and treatment.

Stimuli-Responsive Nanoparticles

Stimuli-responsive nanoparticles, capable of modulating their physicochemical properties in response to external stimuli, have garnered significant attention for controlled drug delivery and release [7]. These nanoparticles can be engineered to respond to various stimuli, such as pH, temperature, light, and enzymatic activity, enabling triggered drug release at the target site [71]. Stimuli-responsive nanoparticles offer precise spatiotemporal control over drug release kinetics, minimizing off-target effects and maximizing therapeutic efficacy.

Targeted Nanoparticle Delivery Systems

Targeted nanoparticle delivery systems, functionalized with ligands that selectively bind to receptors overexpressed on diseased cells or tissues, offer enhanced specificity and efficacy in therapeutic interventions [72]. Ligands, such as antibodies, peptides, and aptamers, can be conjugated to the surface of nanoparticles to facilitate active targeting and internalization into target cells [73]. Targeted nanoparticle delivery systems enable selective accumulation of therapeutic agents at the disease site, minimizing systemic toxicity and improving treatment outcomes.

8.3 Emerging Technologies and Platforms

Emerging technologies and platforms in theranostic nanomedicine hold promise for addressing current challenges and expanding the scope of applications in biomedical research and clinical practice. From novel nanoparticle formulations to advanced imaging techniques, these innovations are reshaping the landscape of theranostic nanomedicine [74].

Organic-Inorganic Hybrid Nanoparticles

Combining the advantages of organic and inorganic materials, organic-inorganic hybrid nanoparticles offer unique properties for theranostic applications [75]. These nanoparticles can be engineered to exhibit synergistic effects, such as enhanced stability, biocompatibility, and imaging contrast [76]. Organic-inorganic hybrid nanoparticles hold promise for various biomedical applications, including cancer therapy, imaging, and regenerative medicine.

Microfluidic-Based Synthesis Platforms

Microfluidic-based synthesis platforms enable precise control over nanoparticle size, shape, and composition, offering scalability and reproducibility for large-scale production [77]. These platforms utilize microscale fluid dynamics to precisely manipulate reaction conditions and particle formation kinetics, leading to uniform and monodisperse nanoparticles [78]. Microfluidic-based synthesis platforms have the potential to streamline the development and optimization of theranostic nanoparticles, accelerating their translation into clinical applications.

3D Bioprinting of Theranostic Constructs

3D bioprinting technology enables the fabrication of complex tissue constructs with integrated theranostic functionalities, offering new opportunities for personalized medicine and regenerative therapy [79]. Theranostic constructs, composed of biomaterials and theranostic nanoparticles, can be precisely engineered to mimic the native tissue microenvironment while enabling real-time imaging and therapeutic interventions [80]. 3D bioprinting of theranostic constructs holds promise for applications in tissue engineering, drug screening, and personalized medicine. Advancements in theranostic nanomedicine, including recent research findings, innovative approaches to nanoparticle engineering, and emerging technologies and platforms, are driving the development of next-generation theranostic agents with enhanced functionality and efficacy.

9 Clinical Translation and Regulatory Considerations

9.1 Challenges in Biocompatibility and Safety

Despite the promising potential of theranostic nanomedicine, several challenges related to biocompatibility and safety must be addressed for successful clinical translation. The complex interactions between nanoparticles and biological systems raise concerns regarding potential cytotoxicity, immunogenicity, and long-term effects on human health [81].

Biocompatibility Assessment

The biocompatibility of theranostic nanoparticles is a critical consideration in their clinical translation. Comprehensive biocompatibility assessments, including in vitro cytotoxicity assays, in vivo toxicity studies, and immunological evaluations, are essential for determining the safety profile of nanoparticle-based therapeutics [82]. Factors such as particle size, surface charge, surface functionalization, and degradation kinetics can influence the biocompatibility of nanoparticles and must be carefully optimized to minimize adverse effects.

Immunological Responses

Nanoparticles have the potential to elicit immune responses, leading to inflammation, immune activation, or hypersensitivity reactions [83]. The immunological properties of theranostic nanoparticles, including their interactions with immune cells, cytokine release profiles, and complement activation pathways, must be thoroughly investigated to assess their immunotoxicity [83]. Strategies for mitigating immunological responses, such as surface modification with biocompatible coatings or immunomodulatory agents, may enhance nanoparticle-based therapeutics' safety profile.

Long-Term Safety Assessment

Long-term safety assessment is crucial for evaluating the potential risks of chronic exposure to theranostic nanoparticles. Studies investigating nanoparticle biodistribution, accumulation, and clearance over extended periods are necessary to assess their long-term effects on vital organs and physiological functions. Preclinical studies employing relevant animal models and rigorous histopathological analyses are essential for identifying potential toxicity endpoints and informing clinical trial design.

9.2 Preclinical and Clinical Studies

Preclinical and clinical studies are pivotal in translating theranostic nanomedicine from bench to bedside, providing critical evidence regarding safety, efficacy, and feasibility in human subjects. Preclinical studies rigorously evaluate nanoparticle pharmacokinetics, biodistribution, toxicity, and therapeutic efficacy in relevant animal models, laying the foundation for subsequent clinical trials.

Preclinical Evaluation

Preclinical evaluation of theranostic nanoparticles encompasses studies that characterize their physicochemical properties, biological interactions, and therapeutic potential. *In vitro* studies use cell culture models to assess cellular uptake, cytotoxicity, and nanoparticle targeting efficiency [84]. *In vivo* studies involve the systemic administration of nanoparticles in animal models to evaluate pharmacokinetics, biodistribution, tumor accumulation, and therapeutic efficacy [85]. Imaging techniques, such as MRI, PET, and optical imaging, enable real-time monitoring of nanoparticle behavior in vivo, providing valuable insights into their pharmacokinetics and biodistribution [86].

Clinical Trials

Following preclinical validation, clinical trials evaluate the safety, efficacy, and feasibility of theranostic nanomedicine in human subjects. Phase I trials focus on assessing nanoparticle safety profile, dose escalation, and pharmacokinetics in healthy volunteers or patients with the target disease. Phase II trials involve preliminary efficacy assessments in patients with the target disease, exploring potential therapeutic benefits, and optimizing treatment regimens [87]. Phase III trials are large-scale, randomized controlled trials designed to confirm therapeutic efficacy, compare treatment outcomes with standard-of-care therapies, and obtain regulatory approval for clinical use [88].

Regulatory Considerations

Regulatory agencies, such as the Food and Drug Administration (FDA) in the United States and the European Medicines Agency (EMA) in Europe, play a crucial role in overseeing the clinical development and approval of theranostic nanomedicine. Regulatory pathways for approval require comprehensive documentation of preclinical data, clinical trial protocols, safety assessments, and manufacturing processes [89]. Successful regulatory approval depends on demonstrating the safety, efficacy, quality, and consistency of theranostic nanoparticles through well-designed preclinical and clinical studies [90]. Collaborative efforts between researchers, clinicians, regulatory agencies, and industry partners are essential for navigating the regulatory pathway and ensuring timely translation of theranostic nanomedicine into clinical practice.

9.3 Regulatory Pathways for Approval

The regulatory approval process for theranostic nanomedicine involves multiple stages, from preclinical development to post-market surveillance, to ensure nanoparticle-based therapeutics' safety and efficacy.

Preclinical Development

Preclinical development of theranostic nanomedicine involves extensive characterization of nanoparticles, including physicochemical properties, stability, biocompatibility, and toxicity. Preclinical studies assess nanoparticles' pharmacokinetics, biodistribution, and therapeutic efficacy in relevant animal models, providing essential data for clinical trial design [91]. Regulatory agencies require comprehensive preclinical data demonstrating the safety and feasibility of theranostic nanoparticles before advancing to clinical trials.

Clinical Trials

Clinical trials are conducted to evaluate the safety, efficacy, and feasibility of theranostic nanomedicine in human subjects, following regulatory guidelines and Good Clinical Practice (GCP) standards [92]. Phase I trials assess safety, tolerability, and pharmacokinetics, while Phase II and III trials investigate therapeutic efficacy and comparative effectiveness. Regulatory agencies review clinical trial protocols, patient consent forms, and data monitoring plans to ensure patient safety and data integrity throughout the trial process [93].

Regulatory Approval

Regulatory approval for theranostic nanomedicine requires the submission of a New Drug Application (NDA) or Biologics License Application (BLA) to the regulatory agency, along with comprehensive documentation of preclinical and clinical data [94]. The regulatory review involves a rigorous assessment of safety, efficacy, quality, and manufacturing processes to determine whether the benefits of the theranostic nanomedicine outweigh the risks [95]. Conditional approval or accelerated pathways may be granted for theranostic nanomedicine addressing unmet medical needs or demonstrating significant therapeutic benefits.

Post-market Surveillance

Post-market surveillance involves continued monitoring of theranostic nanomedicine following regulatory approval to detect adverse events, monitor long-term safety, and assess real-world effectiveness [96]. Pharmacovigilance systems and adverse event reporting mechanisms facilitate ongoing evaluation of nanoparticle safety and inform regulatory decision-making [97]. Collaborative efforts between regulatory agencies, healthcare providers, and industry stakeholders ensure theranostic nanomedicine's continued safety and efficacy in clinical practice.

Clinical translation of theranostic nanomedicine requires addressing challenges related to biocompatibility and safety, conducting rigorous preclinical and clinical studies, and navigating complex regulatory pathways for approval. Collaborative efforts between researchers, clinicians, regulatory agencies, and industry partners are essential for advancing theranostic nanomedicine from bench to bedside and improving patient outcomes in clinical practice.

10 Future Perspectives and Opportunities

10.1 Potential Impact on Healthcare

Integrating theranostic nanomedicine into healthcare is promising for transforming disease management and improving patient outcomes across various medical domains.

Precision Medicine Advancements

Theranostic nanomedicine offers a paradigm shift toward precision medicine by enabling tailored treatment strategies based on individual patient characteristics, disease profiles, and genetic makeup. By integrating diagnostic imaging and therapeutic interventions, clinicians can identify precise molecular targets, predict treatment responses, and optimize therapeutic regimens for enhanced efficacy and reduced side effects [98].

Early Disease Detection and Intervention

One of the most significant advantages of theranostic nanomedicine is its ability to facilitate early disease detection and intervention through integrated diagnostics and therapy [99]. By leveraging advanced imaging modalities and molecular probes, theranostic nanoparticles enable the early detection of disease biomarkers and pathological changes, allowing for timely intervention and disease prevention [100]. Early diagnosis and intervention improve treatment outcomes and reduce healthcare costs associated with advanced disease stages and complications [101].

Enhanced Therapeutic Precision and Efficacy

Theranostic nanoparticles enhance therapeutic precision and efficacy by enabling targeted drug delivery to diseased tissues while minimizing systemic toxicity and off-target effects [102]. Through active targeting mechanisms, nanoparticles can selectively accumulate in diseased cells or tissues, delivering therapeutic payloads with high specificity and efficiency [103]. Real-time monitoring of treatment responses further allows clinicians to adjust therapeutic regimens based on individual patient needs, optimizing treatment outcomes and improving overall patient survival rates.

10.2 Personalized Medicine Approaches

Personalized medicine approaches are poised to benefit significantly from integrating theranostic nanomedicine, allowing for tailored treatment strategies based on individual patient characteristics, genetic profiles, and disease phenotypes.

Genomic and Molecular Profiling Integration

Theranostic nanomedicine facilitates the integration of genomic and molecular profiling data into clinical decision-making processes, guiding personalized treatment selection and optimization [104]. By analyzing genetic mutations, gene expression patterns, and protein biomarkers, clinicians can identify specific molecular targets for theranostic interventions and predict individual treatment responses [105]. Nanoparticle-based imaging modalities provide valuable insights into disease biology and treatment responses, enabling personalized medicine approaches tailored to individual patient needs [106].

Tailored Drug Delivery Strategies

Integrating theranostic nanomedicine enables precise control over drug delivery kinetics, allowing for tailored therapeutic regimens based on patient-specific factors such as disease stage, tumor heterogeneity, and comorbidities [107]. By incorporating diagnostic imaging capabilities, theranostic nanoparticles enable real-time monitoring of drug distribution, pharmacokinetics, and treatment responses, guiding individualized treatment adjustments [108]. Personalized drug delivery systems optimize therapeutic outcomes while minimizing adverse effects and treatment-related toxicity.

Therapeutic Decision Support Systems

Integrating theranostic nanomedicine with digital health technologies, such as AI and ML, facilitates the development of therapeutic decision support systems that analyze complex patient data to inform personalized treatment strategies [109]. By leveraging large-scale genomic, clinical, and imaging datasets, AI-driven algorithms can identify treatment biomarkers, predict treatment responses, and optimize therapeutic regimens in real-time [110]. Therapeutic decision support systems empower clinicians with actionable insights, enabling precision medicine approaches tailored to individual patient needs and preferences [111].

10.3 Integration with Digital Health Technologies

Integrating theranostic nanomedicine with digital health technologies holds promise for advancing disease management, improving patient outcomes, and transforming healthcare delivery [112]. Most of the technologies introduced bring nanoscience close to patient monitoring with much precision, as described below:

Wearable and Implantable Devices

Wearable and implantable devices with theranostic capabilities enable continuous real-time monitoring of physiological parameters, disease biomarkers, and treatment responses [113]. These devices, integrated with theranostic nanoparticles, provide personalized health monitoring and disease management solutions, empowering patients to participate actively in their healthcare. Nanoparticle-based sensors and imaging agents enable non-invasive monitoring of disease progression, treatment efficacy, and patient adherence, facilitating timely intervention and personalized care.

Telemedicine and Remote Patient Monitoring

Telemedicine platforms and remote patient monitoring systems leverage theranostic nanomedicine to enable virtual consultations, remote diagnostics, and home-based treatments [114]. Nanoparticle-based imaging and sensing technologies facilitate remote data collection, analysis, and transmission, enabling healthcare providers to monitor patient health status, track treatment responses, and adjust therapeutic regimens [81]. Telemedicine interventions improve access to healthcare services, reduce healthcare disparities, and enhance patient engagement and satisfaction [115].

Data Analytics and Predictive Modeling

Data analytics and predictive modeling techniques harness the information generated by theranostic nanomedicine to identify disease trends, predict patient outcomes, and optimize healthcare delivery [116]. By analyzing large-scale datasets, including genomic, clinical, and imaging data, predictive models can identify disease biomarkers, stratify patient populations, and guide personalized treatment decisions [116]. Theranostic nanoparticles play a critical role in data acquisition, providing high-resolution imaging, molecular profiling, and real-time monitoring capabilities for disease management [117].

11 Conclusion

Theranostic nanomedicine represents a transformative approach at the intersection of diagnostics and therapy, offering unprecedented opportunities for personalized and precise healthcare. The implications of theranostic nanomedicine for healthcare practice and policy are profound. Theranostic approaches can improve patient outcomes, reduce healthcare costs, and revolutionize disease management across various medical disciplines by providing personalized treatment strategies, early disease detection, and enhanced therapeutic efficacy. In healthcare practice, integrating theranostic nanomedicine may necessitate the development of new diagnostic and therapeutic protocols and specialized training for healthcare providers to utilize these technologies effectively. Moreover, healthcare policies may need to adapt to accommodate the regulatory complexities and ethical considerations associated with theranostic nanomedicine, ensuring patient safety, accessibility, and affordability. Theranostic nanomedicine represents a frontier in healthcare innovation, offering tailored treatment strategies, early disease detection, and enhanced therapeutic efficacy. While challenges remain, the future of theranostic nanomedicine is bright, with opportunities for transformative impact on patient care, healthcare practice, and policy.

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Navigating the Nanoscale Frontier: An In-Depth Introduction to the World of Nanomedicine

Pranav Bhaskar, Rashmi Tripathi, Swati Singh, Amrit Chattopadhaya, Ashish Verma, and Vibhav Gautam

1 Introduction

Nanomedicine is an interdisciplinary research area involving the design, development, and use of nanoscale structures and devices for the therapeutic, diagnostic, and prevention of many diseases. Nanotechnology manipulates the unique physical, chemical, and biological properties of nanomaterials which are known to be different from their bulk form. At the nanoscale level, the materials are enriched with extra features such as more reactivity, better biocompatibility, and engaging biological systems at the molecular level [1]. They are key to the creation of diagnostic kits, targeted treatment systems, and regenerative therapies, as well as hurting other applications.

The discipline of nanomedicine has come up as a groundbreaking field where nanoscience and medicine meet at the crossroads, bringing an opportunity to rethink healthcare from a classical point of view. Recently, the capability of humans to alter and dominate the properties of matter at the nanoscale level has become the platform for the exploration of new ways of developing advanced diagnostic and therapeutic means [2]. Nanomedicine derives its stronghold from fusing various

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disciplines such as materials science, biology, chemistry, engineering, and medicine. The more that the researchers investigated the properties and behavior of the materials at the nanoscale, the more they discovered that some special phenomena and characteristics could be used in various clinical applications like disease diagnosis and therapeutics.

The importance and future of nanomedicine existence is based on the fact that nanotechnological approaches are the right way to solve some of the prominent problems in modern medicine. Among the main directions, nanomedicine has a chance to realize itself intentionally is in the field of targeted drug delivery into a tumor [3]. As for traditional drug formulation processes, their poor specificity is the cause of the side effects that are not desired and off-target. Nanomedicine offers the means that drug compounds can be loaded into nanocarriers such as liposomes or polymeric nanoparticles, these nanocarriers can be aimed precisely at the designated therapeutic site, and in this, the chance of toxicity and improving the therapeutic potency is minimized [4].

Developing cutting-edge technology for highly sensitive diagnostic devices is another area where nanomedicine comes to the forefront. The nanoparticles—especially the nanoparticle-based contrast agents and biosensors—have shown the ability to enhance the sensibility and the precision of the imaging techniques so that early and accurate disease detection becomes possible [5]. On top of that, they can be manufactured to react with specific biomolecules through which scientists can identify new methods of building highly specific and sensitive diagnostic tools.

Alongside the areas of diagnostics and therapeutics, nanomedicine also has regenerative potential for the world of health. Nanoparticles can mimic the naturally occurring extracellular matrix and can serve as a base for bioengineering processes and regenerative healing procedures [6]. Moreover, the bringing together of nanomaterials with stem cell therapies and genome editing technologies could be what makes for the next frontier of the regenerative therapies that can be used for various diseases and injuries.

2 Fundamental Concepts in Nanoscience

In a bid to understand how nanomedicine could be transformational, it is necessary that we first comprehend the basic concepts that dictate the behavior of materials at that level. Among the major principles, there is the idea that size and scale are affected by several environmental factors. The nanoscale level of material (1–100 nm) is where one starts to observe quantum effects and other physicochemical properties that are very different from the ones observed at the bulk scale [7]. Materials in the nanoscale dimension, for example, nanoparticles, nanotubes, and nano-shells, hold a large surface-to-volume ratio, and therefore are more reactive, which fosters their better interactions with biological bodies [7, 8]. Next, a quantum phenomenon at the nanoscale creates unusual optical, electronic, and magnetic features, helping the making of better diagnostic and treatment machines [9].

2.1 Size and Scale

Nanoscience is all about phenomena happening on the tiniest scales unit between 1 nanometer (1 nm) and 100 nanometers (100 nm). A nanometer is 1 billionth of a meter, hence, absolutely small compared to the usual figures we tend to see and imagine. However, after the reduction of the materials to such tiny atomic scales, they demonstrate unique properties and behaviors that are quite opposite to how these materials behave in their conventional form [10].

2.2 Nanomaterials and Their Properties

Nanomaterials are materials that exceed a minimum of one of their dimensions to be considered as nanomaterials; for instance, nanoparticles, nanotubes, nanowires, and nanofilms. The small size of nanomaterials translates into their high ratio of surface-to-volume, improved reaction ability, enhanced solubility, and specific optical, electric, and magnetic properties [7]. One of the unique features of nanoproducts is their aptness to establish interactions with biological systems on the level of molecules, cells, and tissues [8, 11]. Such targeted nature of the nanoparticles makes them valuable for treatments requiring direct delivery of therapeutic agents, bioimaging, and sensing.

2.3 Quantum Effects at the Nanoscale

At this scale, quantum effects have a higher chance of making influence, and materials' behavior depends on them. Consequently, these quantum effects occur because of the lack of space for their electrons to move around at such a minute level and because of the wavelike nature of matter. The quantum effect in which the material in the nanoscale ratio behaves differently than at the larger scale is known as the quantum confinement effect. This could result in an alteration in the electrocatalysis of the material which would be valuable in optoelectronics, energy storage, and catalysis [7, 12].

Besides this phenomenon at the nanoscale, there is an effect that occurs in conjunction with the tunability of optical properties as well. Nanoparticles can demonstrate remarkable optical attributes that make it possible to tune the surface plasmon effect simply by variation in the size, shape, and composition of the nanoparticles [13]. This response is essential in revealing the application of the medical field which involves biosensing, imaging, and phototherapy. It is therefore of great importance for nanotechnology to be able to forge a deep understanding of these basic nanoscale phenomena which are key to bringing forth nanomaterials being applied in medicine, energy, electronics, and catalysis. The peculiar characteristics and actions of the materials at the nanoscale offer endless possibilities for the development of novel and revolutionary technologies.

3 Nanoparticles in Medicine

Nanoparticles, due to their distinctive physicochemical characteristics, have lately been introduced as effective tools in medicine with the main emphasis on diagnostics, treatment, and drug delivery. These nanomaterials, which are sized between 1 and 100 nm, have unique properties allowing them to be immensely useful in biomedical applications.

3.1 Types of Nanoparticles

Nanoparticles used in medicine can be broadly categorized into organic and inorganic nanoparticles, each with its own set of advantages and applications.

Organic Nanoparticles

These nanoparticles are highly applicable in bioanalysis and to study various molecular behavior of processes like signal transduction, molecular recognition and protein-mediated membrane fusion which involve DNA or antibodies as catalytic interfaces. Organic nanoparticles are typically composed of biocompatible polymers such as poly(lactic-co-glycolic acid) (PLGA), chitosan, or dendrimers [14]. These nanoparticles are often used for drug delivery due to their biodegradability and ability to encapsulate a wide range of therapeutic agents.

Inorganic Nanoparticles

Inorganic nanoparticles include materials such as gold, silver, iron oxide, and silica and these nanoparticles exhibit unique optical, magnetic, and electronic properties that make them valuable for various biomedical applications, including imaging, drug delivery, and therapy [15]. Several reports are available for the application of synthesized nanoparticle and their role in disease management [99]. In one of the studies, mycogenically synthesized silver nanoparticle from the aquous extract of *Penicillium oxalicum* exerts cytotoxic potential against two different breast cancer cells i.e., MDA-MB-231 and MCF-7 [41]. Another report has validated the cytotoxic potential of bio fabricated silver nanoparticle from the aquous extract of *Colletotrichum gloeosporiodes* against breast cancer cells [100]. In a recent report, mycogenic gold nanoparticle exhibited significant anticancer activity against two different human breast cancer cells such as MCF-7 and MDA-MB-231 [101].

3.2 Synthesis and Functionalization

The synthesis of nanoparticles involves the production of nanoscale materials with controlled size, shape, and surface properties. Various methods, such as chemical precipitation, sol-gel synthesis, and emulsion techniques, are employed to produce nanoparticles with desired characteristics [16].

Chemical Precipitation

In this method, nanoparticles are synthesized by precipitating the desired material from a solution through chemical reactions. For example, gold nanoparticles can be synthesized by reducing gold salts in the presence of a stabilizing agent.

Sol-Gel Synthesis

Sol-gel synthesis involves the formation of nanoparticles through the hydrolysis and condensation of precursor molecules in a liquid solution. This method is commonly used to produce silica nanoparticles with tailored properties.

Emulsion Techniques

Emulsion techniques, such as the double emulsion method, are used to encapsulate hydrophobic drugs within the core of nanoparticles. This method enables the efficient loading and controlled release of therapeutic agents.

Functionalization of nanoparticles involves modifying their surface properties to enhance biocompatibility, stability, and targeting capabilities. Surface functionalization can be achieved through the attachment of various biomolecules, such as antibodies, peptides, or targeting ligands, to the nanoparticle surface [17].

3.3 Biocompatibility and Toxicity Considerations

The biocompatibility of nanoparticles is a crucial consideration for their safe use in biomedical applications. While nanoparticles offer numerous benefits, they can also pose potential risks to biological systems due to their small size and unique properties.

Biocompatibility

Biocompatible nanoparticles are designed to minimize adverse effects on biological systems, ensuring compatibility with living tissues and cells. Surface modification of nanoparticles with biocompatible polymers, such as polyethylene glycol (PEG), can enhance their biocompatibility and reduce immune responses [18, 19].

Toxicity Considerations

Despite their potential benefits, nanoparticles can exhibit toxicity depending on their size, shape, surface charge, and composition. Factors such as nanoparticle accumulation, cellular uptake, and interactions with biological molecules can influence their toxicity [20]. Therefore, a thorough evaluation of nanoparticle toxicity is essential to ensure their safe use in medicine.

Nanoparticles represent a promising platform for revolutionizing healthcare through their diverse applications in diagnosis, therapy, and drug delivery. With ongoing research focused on enhancing their biocompatibility, stability, and targeting capabilities, nanoparticles hold great potential for addressing the complex challenges in modern medicine and improving patient outcomes.

4 Diagnostic Applications of Nanomedicine

Diagnostics have been greatly improved through nanomedicine since some new techniques and strategies provide early diagnosis of diseases at the molecular level with minimal levels of interference. So, with the help of the properties that are characteristic of nanomaterials, specialists have created effective imaging methods with a high level of sensitivity and accuracy, biosensors, nano sensors, and diagnostics based on nanomaterials (Fig. 3a), which are changing the industry.

4.1 Imaging Techniques

Nanotechnology has so greatly benefited imaging, and daily improvements are being made to the quality of images of the body's tissues and functions. Some of the key nanoparticle-based imaging techniques include:

Magnetic Resonance Imaging (MRI)

The integration of magnetic resonance imaging (MRI) and nanoparticles has significantly advanced medical diagnostics by providing a universal approach to enhance imaging capabilities. Nanoparticles, especially those composed of iron oxide, gadolinium, or manganese, have unique magnetic properties that are used for targeted imaging. The unique compatibility of nanoparticles with the body and their ability to degrade over time are fundamental advantages when used in MRI. To improve the accuracy of diagnosis functional modification of nanoparticles approach is adopted which increases the sensitivity by reducing the background noise and minimizing non-specific interaction. The nanoparticles used in heteronuclear MRI are typically made of polymer and (or) lipid, with the heteronuclear MRI atoms incorporated in the polymer and lipid. Because of their hydrophobic qualities, these agents frequently produce self-assembling nano-emulsions in aqueous environments. Iron oxide nanoparticles include superparamagnetic nanoparticles (SPIO), magnetic iron oxide nanoparticles (MION) and cross-linked iron oxide nanoparticles are very useful in MRI because they enhance the visibility of tumors, inflammation, and abnormal blood vessels [21]. These nanoparticles improve the tissue's magnetization and therefore improve the contrast images. Some other examples involve the development of 19F-MRI nanoparticles (PLGA-PFCE-ICG nanoparticles, HBPFPE-aptamer) from highly fluorinated compounds like perfluorocarbons, and perfluorinated crown ethers which are used for breast cancer detection [103]. The clinically approved gadolinium (Gd3+) complexes on conjugation with high-density lipoprotein (HDL) helps in the bioimaging of atherosclerotic plaque of heat arterial walls [44].

Computed Tomography (CT)

Computed tomography (CT) can exhibit a higher spatial-temporal resolution of diagnosis which includes X-ray attenuation of differential tissue to create 3-D images. Conventional CT involves iodine as a contrasting agent which helps in distinguishing the differential tissue images amongst tissue with similar attenuation coefficient. This approach has limitations like a greater chance of ionizing radiation exposure, renal toxicity, etc. To minimize this, nanosized contrasting agents were developed which increases the accuracy avenue of CT imaging. The iodine-based contrast agents were loaded in classical core-shell nanoparticles encapsulated in liposomes in which nanoparticles serve as vectors, giving rise to iodine-based liposomal technology which helps reduce the iodine-based toxicity. Another group involves metal-based contrasting agents which involve tantalum oxide [104], zirconium dioxide [45] and gold as nanoparticles [46]. Gold nanoparticles have most commonly been used in CT analysis because these nanoparticles possess high atomic numbers, which enable them to strongly interact with X-rays [22]. This makes it even easier to visualize vessels and growths such as tumors. AuNPs conjugated with prostate-specific membrane antigen (PMSA) specific aptamer has been reported for imaging of prostate cancer [48].

Fluorescence Imaging

The fluorescence imaging approach provides the spatial resolution at its maximum level of accuracy for diagnosis at the microscopic level. It has much more capability in deeper tissue penetration and minimum non-specific tissue auto-fluorescence. Nanoparticles can help overcome the limits of fluorescence imaging. The most commonly used nanoparticle for better fluorescence imaging involves surface-labeled fluorophore as vectors which includes magnetic nanoparticles bound with Cy3-modified S6 aptamer, which increases the imaging ability of fluorophore via active and passive targeting by enabling its local concentration [49]. Quantum dots like (QD710-Dendron) are semiconductor particles of dimensions of nanometers with distinctive photophysical characteristics that include size-dependent fluorescence [50]. Quantum dots offer broad absorption spectra and controllable fluorescence emission. Quantum dots use FRET, with a significant Stokes shift resulting in long wavelengths that increase tissue penetration depth and diminish background autofluorescence. It applies to fluorescence imaging which is employed in staining biomolecules or cells or certain tissues to produce high-resolution and sensitivity images [23]. Nanoparticles used in fluorescence imaging also have potential application in the field of protein analysis, gene identification, evaluation of enzyme activity (Cy5.5-DEVD-DOPAK/AuNP) [52], cell tracking and diagnosis of disease and tumor monitoring assessment (PLNP(Zn1.1Ga1.8Ge0.1O4:Cr3+)-CuS-RGD).

Photoacoustic Imaging (PAI)

In Photoacoustic Imaging (PAI), the focus lies on the interaction between laser-induced ultrasound and the light-absorbing properties of nanoparticles. When subjected to pulsed laser light, nanoparticles take in energy and emit ultrasonic waves, allowing for the generation of intricate images. The versatility of nanoparticles in PAI supports molecular targeting, aiding in the detection of biomarkers and early diagnosis. Combining PAI with nanoparticles presents a powerful approach for cancer theragnostic. The high-quality, real-time images produced by PAI have enhanced cancer diagnosis, monitoring, and treatment [53]. PAI has also been utilized to observe the transport and activation of therapeutic genes carried by nanoparticles [54]. Gold nanorods and carbon nanotubes are employed in photoacoustic imaging that combines the optical and ultrasonic forms of imaging. They can collect light and generate sound waves and consequently apply real-time tissue images in deeper tissues [55].

4.2 Biosensors and Nanosensors

Biosensors and nano sensors are identification instruments used for the qualitative and quantitative identification of biomolecules based on the incorporation of a biological recognition structure with a transducer. Nanomaterials improve the detection rate and accuracy of these sensors through efficient disease diagnosis at early stages.

Electrochemical Biosensors

These types of biosensors work on the principle of electrochemical reaction on a transducer surface between receptors and detectable analytes which provide label-free detection along with high signal-to-volume ratio, leads to the detection of biomolecules non-invasive way. Gold nanoparticles, silver ones, and carbon nanotubes can be used for the electrode surface modification of electrochemical biosensors. These changes enhance the surface area and facilitate electron transfer, and thus higher sensing capabilities of biomolecules of interest glucose, DNA, and proteins. Use of carbon nanotubes as the immobilization matrix are used for glucose oxidase and H_2O_2 [56]. Another group developed a wearable electrochemical biosensor for high glucose sensitivity by the use of (reduced graphene oxide) rGO/polyurethane modified with functional groups consisting of oxygen. For analysis of the level of cholesterol bio enzymes like cholesterol esterase and cholesterol oxidase were immobilized with the utilization of nanomaterials like $NiFe_2O_4$, Tm_2O_3 and Cu_2O as electrode materials [57].

Optical Biosensors

Optical biosensors are the most versatile type of biosensors which involve optical transducer to analysis the bio analyte and convert the data into electrical signal. They possess high potential for analysis because of their high selectivity and rapid and extremely high sensitivity measurements. Optical biosensors utilize plasmonic nanoparticles, specifically gold and silver nanoparticles. Target molecules are detected by these sensors by variations in the optical characteristics of nanoparticles, such as surface plasmon resonance (SPR). Biomolecular interactions are monitored in real-time using SPR-based biosensors [55]. Some other examples of optical biosensors include use of titanium oxide (TiO_2) and aluminum oxide (Al_2O_3) as dielectric films which are combined with fibre-optic-fabry-Perot interferometer used for estimation of hemoglobin and glucose [58]. This interferometer on combination with polydimethylsiloxane (PDMS) fabrications are useful for lipidomics profiling . Polymer-based two-dimensional photonic crystal film has been used for the detection of C-reactive protein produced by the liver [59].

Nanosensors

Nanosensors utilize the unique properties of nanomaterials to identify chemical alterations at the nanoscale. Nanowires, graphene, and carbon nanotubes are frequently used in nanosensors because of their superior electrical conductivity and large surface area. Due to the ability to identify biomarkers at low concentrations, diseases including cancer and infections can be diagnosed early.

4.3 Nanomaterial-Based Diagnostics

Nanomaterials are incorporated into diagnostic assays to enhance their performance to offer rapid, accurate, and affordable disease detection.

Lateral Flow Assays (LFAs)

The lateral flow assay involves moving a liquid sample with the analyte of interest down a strip. The strip is constructed of polymeric materials and includes specific zones or places where molecules have been coupled with labels. When an analyte-containing sample travels through these zones, it interacts with molecules that bind particularly to the analyte. A common point-of-care diagnostic test is the lateral flow assay, which makes extensive use of gold nanoparticles [60]. The presence of target analytes is indicated visually by these nanoparticles in the form of colorful lines. Pregnancy hormones, infections, and other indicators are all detected with LFAs.

Lab-on-a-Chip Devices

Lab-on-a-chip devices use nanomaterials to combine several diagnostic capabilities into a single platform while also miniaturizing the devices. These devices carry out intricate diagnostic procedures with small sample quantities and excellent precision by using nanoparticle-based chemicals and microfluidic channels. A diagnostic system which include the combination of quantum dots and microfluidics resulted in a highly sensitive analysis tool for blood borne infectious disease like HIV, hepatitis B, hepatitis C [61].

Nanoparticles-Based PCR

The combination of polymerase chain reaction (PCR) with nanoparticles and nanomaterials have given rise to a potential tool for ultrafast nucleic acid amplification. By enhancing the heat conductivity and reaction kinetics, nanoparticles improve PCR experiments [62]. The nanoparticle centric PCR involves the use of nanoparticles and nanomaterials like graphene (GO) [63], carbon nanotubes, gold, silver, titanium oxides, quantum dots (e.g. thiacetic acid modified CdTe and thioglycolic acid modified CdSe and CdSe/ZnS) [64], polyamide amine dendrimers coupled with gold nanoparticles (PAMAM-G5-NH_2-AuNp [65]. This facilitates the quicker and more precise amplification of genetic material, which helps identify genetic abnormalities and infectious diseases [66].

Advanced imaging methods, extremely sensitive biosensors and nanosensors, and cutting-edge nanomaterial-based diagnostics have all been made possible by nanomedicine, revolutionizing diagnostic applications. By enabling early and precise illness detection, these technologies open the door to prompt therapies and better patient outcomes. We can anticipate other developments that will further alter the field of medical diagnostics as long as research in nanomedicine advances.

5 Therapeutic Nanosystems

Therapeutic nanosystems provide accurate and efficient treatments for a variety of medical problems by utilizing the special qualities of nanoparticles to transform current treatment approaches. Innovative methods for drug administration and targeted therapies, regenerative medicine, theranostics, and controlled release strategies are all included in these systems, and they all significantly improve patient care.

5.1 Drug Delivery and Targeted Therapies

In the areas of targeted therapy and drug delivery, nanotechnology is having one of the biggest effects on medicine. Therapeutic drugs can be delivered more efficiently and with minimal side effects by engineering nanoparticles.

Nanoparticles for Drug Delivery

The nano-based drug delivery system (NDDs) in cancer involveinvolves two modes of targeting a) passive tumor targeting b) active tumor targeting which generally based on a phenomenon in tumor cells known as enhanced permeation and retention effect (EPR). The chemotherapeutic drugs were uncoated in the PEGylated NDDs with improved hydrophilicity and minimize immunogenicity show stability in the blood circulation and evade mononuclear phagocyte system clearance so that they can achieve maximum EPR effect and reaches the tumors, in case of passive targeting. In active tumor targeting, the NDDs affinity with the cancer cell surface receptors enhance their uptake into cancer cell through receptor mediated endocytosis. This NDDs also modulate tumor microenvironment in active targeting [67]. Drugs are encapsulated in nanoparticles which are categorized as inorganic based NDDs include metallic nanoparticles [68], mesoporous silica nanoparticles, and organic based NDDs includes carbon-based nanocarriers, dendrimers (e.g- 5-aminolevulinic acid-containing (ALA) dendrimer, poly-L-lysine (PLL) dendrimers) [69], lipid-based nanocarriers (e.g.- liposome-formulation with doxorubicin for Kaposi's sarcoma, cytarabine for lymphomatous meningistis, niosomal nano formulation of cisplatin , tamoxifen citrate for breast cancer [70]), polymer based nanocarriers, protein based nanocarriers.liposomes, dendrimers, and polymeric nanoparticle which shield the medications from deterioration and guarantee a controlled release at the intended location. This lowers systemic toxicity while increasing medication absorption and therapeutic index [71].

Targeted Therapies

Antibodies, peptides, or small molecules that selectively bind to receptors on the surface of target cells—like cancer cells—can be used to functionalize nanoparticles. By protecting healthy tissues and minimizing side effects, this tailored strategy guarantees that the therapeutic medicines are administered directly to sick cells. Chemotherapeutic medicines can be precisely delivered to tumor cells by the use of antibody-conjugated nanoparticles [71].

5.2 Nanomaterials in Regenerative Medicine

By increasing the effectiveness of tissue engineering and stem cell therapies, nanomaterials play a critical role in the field of regenerative medicine, which seeks to replace or repair damaged tissues and organs.

Scaffolds for Tissue Engineering

Scaffolds resembling the extracellular matrix are made with nanomaterials, offering structural support for tissue regeneration and cell proliferation. In order to support cell adhesion, proliferation, and differentiation to aid in the regeneration of bone, cartilage, and other tissues, nanofibers, hydrogels, and nanocomposites have been developed [72].

Stem Cell Delivery

Healing and restoring damaged or missing tissues involve intricate interactions among stem cells, growth factors, and the extracellular matrix. Scaffolds that resemble the extracellular matrix need to be created to provide the shape and framework of the organ or tissue being regenerated. These scaffolds must maintain physical stability at the implantation site, facilitate the migration, growth, and maturation of stem cells, and be non-toxic and biodegradable. It is possible to use nanoparticles to improve the engraftment and delivery of stem cells to wounded tissues. Regenerative therapies can be made more effective by utilizing magnetic nanoparticles, which can be utilized to direct stem cells to specific locations through the application of an external magnetic field. According to the study, RADA16 peptide nanofiber material self-organizes into scaffolds that facilitated the growth of hippocampal neurons, which then generated neurites and synapses [73]. In case of cartilage regeneration, the mesenchymal stem cells (MSCs) were fused with nanoscaffolds of poly-(L-lactic acid) and multi-walled carbon nanotubes (MWNTs). The use of nanofibers made of polyethylene glycol, polycaprolactone and polyurethane along with the embryonic stem cells (ES cells) scaffolds helps in inducting the production of cardiomyocytes from ES cells. The production of dopaminergic neurons from the differentiation of human ES cells cultured on electro spun fibrous polyurethane scaffolds without requirement of growth factors or hormones were also reported.

5.3 Theranostics

Precision and personalized treatment are made possible by theranostics, an integrated method that integrates therapeutic and diagnostic activities into a single nanosystem.

Multifunctional Nanoparticles

Theranostic nanoparticles are intended to serve multiple purposes, including therapeutic and imaging. For instance, gold nanoparticles can be used in imaging, such as CT or MRI, to improve contrast, as well as photothermal therapy, which uses light converted into heat to destroy cancer cells [74].

Real-Time Monitoring

Real-time monitoring of disease progression and therapy efficacy is made possible by theranostic systems. This optimizes therapy outcomes by allowing practitioners to modify treatment procedures based on the patient's response [75].

5.4 Controlled Release Strategies

Therapeutic substances are delivered via controlled release systems at a predefined rate, guaranteeing long-lasting and efficient treatment.

Stimuli-Responsive Nanoparticles

These nanoparticles react to particular stimuli like pH, temperature, or light by releasing their payload. To ensure targeted treatment, pH-sensitive nanoparticles, for instance, can release drugs in the acidic environment of a tumor [76].

Polymeric Micelles and Hydrogels

These nanocarriers use diffusion or degradation processes to release medications over an extended period. Hydrophobic drugs can be encapsulated in polymeric micelles and released gradually over time, whereas hydrogels can be designed to break down in response to biological signals, allowing for controlled drug release [77].

Therapeutic nano systems, which offer cutting-edge approaches to drug delivery and targeted therapies, regenerative medicine, theranostics, and controlled release techniques, constitute a substantial breakthrough in contemporary medicine. Personalized healthcare is made possible by these nano systems, which also reduce adverse effects and improve therapeutic efficacy and precision. Therapeutic nano systems have significant promise for tackling difficult medical challenges and improving patient outcomes as nanotechnology research and development continues.

6 Applications of Nanomedicine in the Treatment of Diseases

Through increased targeting capabilities, decreased side effects, and higher efficacy, nanomedicine is revolutionizing the treatment of a wide range of diseases. This method is being used in many different medical specialties, such as orthopedics, neurological illnesses, cancer treatment, cardiovascular diseases, and infectious diseases (Figs. 1 and 2).

6.1 Cancer Therapy

The field of nanomedicine has had a profound impact on cancer treatment by offering novel approaches to medication delivery, focused therapy, and diagnosis (Fig. 1a).

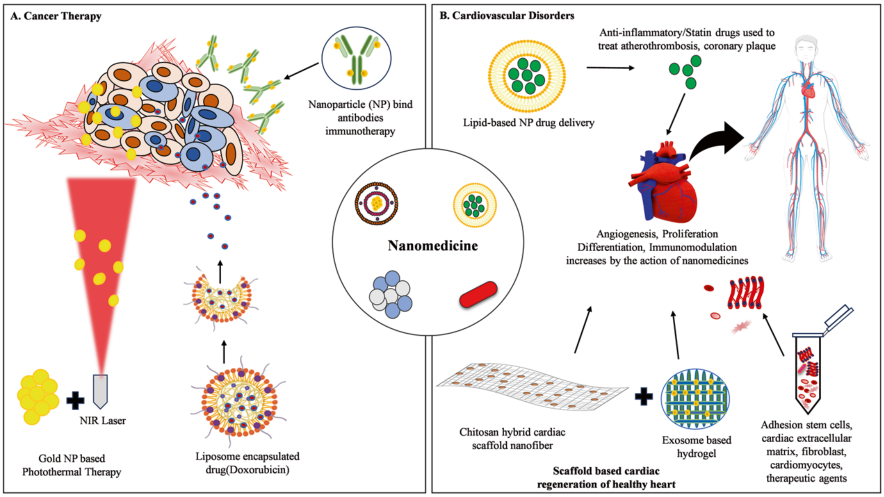
A. Cancer Therapy
Nanoparticle (NP) bind antibodies immunotherapy
NIR Laser
Gold NP based Photothermal Therapy
Liposome encapsulated drug(Doxorubicin)
Nanomedicine
B. Cardiovascular Disorders
Anti-inflammatory/Statin drugs used to treat atherothrombosis, coronary plaque
Lipid-based NP drug delivery
Angiogenesis, Proliferation Differentiation, Immunomodulation increases by the action of nanomedicines
Chitosan hybrid cardiac scaffold nanofiber
Exosome based hydrogel
Adhesion stem cells, cardiac extracellular matrix, fibroblast, cardiomyocytes, therapeutic agents
Scaffold based cardiac regeneration of healthy heart

Fig. 1 The diagrammatic illustration represents the application of nanomedicine in different avenues of medical science. (**a**) The nanomedicine approach is very much efficient and provide great results in the field of cancer therapy by improvising the release of chemotherapeutic agents using liposome and polymeric nanoparticles or via nanoparticle bind antibodies (immunotherapy), also enhanced the photothermal therapy using near infrared region (NIR) laser. (**b**) In the case of cardiovascular diseases, lipid based NP drug delivery system gave better results in treatment of atherothrombosis, coronary plaque in the heart. Scaffold based cardiac regeneration using chitosan-based scaffold nanofiber, exosome-based hydrogels along with the delivery agents (cardiac extracellular matrix, adhesion stem cells, fibroblast, cardiomyocytes, therapeutic drugs) helps to proliferate, differentiate, promote angiogenesis leading to regeneration of healthy heart

Targeted Drug Delivery

Chemotherapeutic medications can be delivered to tumor cells directly by nanoparticle engineering, reducing harm to healthy organs. Drugs like doxorubicin, for instance, can be precisely released at the tumor site via liposomes and polymeric nanoparticles, increasing therapeutic efficacy and lowering systemic toxicity [24, 25]. By preventing phagocyte uptake and encouraging their recruitment to and accumulation within tumours, the addition of polyethylene glycol (PEGylation) or other polymers to the surface of nanoparticles prolongs the duration of these substances' circulation [78, 79]. Unfortunately, the tumour cells themselves are unable to absorb drugs derived as a result of these alterations. "Active" tumor-targeting strategies that attach tumor-specific ligands to the surface of nanoparticles have been developed to get around this restriction. Enhancing the transport and uptake of nanoparticles by tumour cells can be achieved by coating the surface of the particles with ligands for tumour receptors, such as herceptin, folate, or transferrin. The clinical importance of these techniques is still unknown, despite intriguing results from recent studies in animal models employing peptides as brain-tumor-targeting ligands.

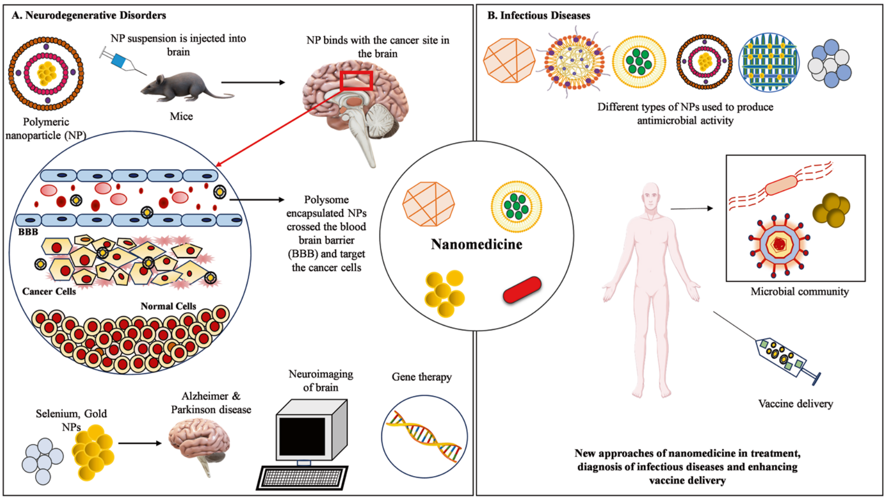
A. Neurodegenerative Disorders
NP suspension is injected into brain
NP binds with the cancer site in the brain
Polymeric nanoparticle (NP)
Mice
BBB
Polysome encapsulated NPs crossed the blood brain barrier (BBB) and target the cancer cells
Cancer Cells
Normal Cells
Nanomedicine
Neuroimaging of brain
Gene therapy
Alzheimer & Parkinson disease
Selenium, Gold NPs
B. Infectious Diseases
Different types of NPs used to produce antimicrobial activity
Microbial community
Vaccine delivery
New approaches of nanomedicine in treatment, diagnosis of infectious diseases and enhancing vaccine delivery

Fig. 2 The diagrammatic illustration represents the application of nanomedicine in different avenues of medical science. (**a**) Brain cancer have become the potential target site of the nanoparticles (Polymeric NPs) which improvise the targeted drug therapy by crossing the blood brain barrier (BBB) and kills the cancerous cell. Nanoparticles of selenium and gold helps in diagnosis of the neurodegenerative disorders like Alzheimer, Parkinson diseases and gave new insights in gene therapy and neuroimaging. (**b**) Nanomedicine offers innovative approaches to prevent, diagnose, and treat infectious diseases. Strong antimicrobial properties make nanoparticles useful for coating medical devices and in wound dressings to stop infections. Additionally, they can be used in formulations to treat fungal, viral, and bacterial illnesses. By improving the stability and delivery of the antigen, nanoparticles are used to create more effective vaccinations. Lipid nanoparticles, for instance, are employed in mRNA vaccines, like the ones created for COVID-19, to shield and transport the mRNA to immune cells

Photothermal and Photodynamic Therapy

In photothermal therapy, gold nanoparticles and other nanomaterials are utilized to specifically kill cancer cells by converting light into heat. Nanoparticles are employed in photodynamic therapy to deliver photosensitizing chemicals, which when activated by light, form reactive oxygen species that kill cancer cells. Since gold nanoparticles have a surface plasmon resonance (SPR) effect that has a high efficiency of converting light to heat and a simple thiolation chemistry for the functionalisation of desired molecules, which enhances their capability to load photosensitiser drugs, they have been considered for use as both photothermal agents and photosensitiser carriers [80]. According to recent research, photothermal (PTT) and photodynamic (PDT) therapies that react to near-infrared (NIR) wavelength lasers by combining fluorescent photodynamic drugs with multifunctional plasmon nanoparticles can work in concert to treat tumours [81, 82]. Because the majority of photosensitisers (PS) are hydrophobic, their therapeutic effects on tumours must be achieved through a delivery method. The majority of work has gone into creating dual PTT and PDT therapies, which use photosensitisers and a variety of gold nanoparticle forms to completely eradicate tumours.

Immunotherapy

By delivering immune-modulating substances that heighten the body's immunological response against tumor cells, nanoparticles are used to improve cancer immunotherapy. For example, vaccines based on nanoparticles can expose the immune system to tumor antigens, enhancing its capacity to identify and combat cancerous cells [26]. In addition to having significant side effects and a high recurrence risk, traditional chemotherapy and radiation therapy have poor therapeutic benefits against cancer. With the encouraging outcomes that immunotherapeutic drugs have recently produced in the clinical treatment of cancer, cancer patients and physicians around the world have become very interested in these agents [83, 84]. Among its many benefits is that cancer immunotherapy can stop metastases and recurrences in addition to treating primary cancer [85]. Biomaterial-based nanoparticles have been employed in recent times to overcome the shortcomings of current cancer immunotherapies [86–88]. Because of their capacity to effectively carry medications to target regions, shield medications from endogenous enzymes, and last extended periods of time in circulation, nanoparticles have been the subject of much research in the field of drug delivery.

6.2 Cardiovascular Diseases

Nanomedicine offers promising approaches for the diagnosis, treatment, and prevention of cardiovascular diseases (CVDs) (Fig. 1b).

Targeted Drug Delivery

Drugs can be delivered to atherosclerotic plaques directly using nanoparticles, which lowers inflammation and stops plaque rupture. Lipid-based nanoparticles can encapsulate anti-inflammatory or statin medications and deliver them to vascular tissues that are sick [27]. Large-scale clinical studies and experimental findings are brought together by theranostic application of nanoparticles in CVDs. By making it possible to develop therapeutic drug delivery systems based on imaging, theranostics in cardiology combines imaging with therapeutics. Various nanoparticles are used in drug delivery, diagnostic imaging, and further evaluation of therapeutic efficacy. Reduces local and systemic effects by controlling the drug delivery via nanoparticles and its action at the target region with light, an external magnetic field, or ultrasonic [89]. Specific sites have been targeted by the use of magnetic resonance imaging for vascular intervention. According to reports, inflammatory processes in atherosclerotic plaques are recognised and inhibited by metallic nanoparticles drawn to the magnetic field [90]. Natural compound-coated magnetic nanoparticles are effectively used in CVD imaging. As an example, fucoidan, a polysaccharide-coated ultra-small superparamagnetic iron oxide nanoparticles have been employed for molecular imaging of arterial thrombus using MRI. In a rat model of elastase-induced vascular injury, this contrasting agent was able to image P-selection, an adhesion molecule recognised as the molecular element of athero-thrombotic illness [91].

Imaging

Cardiovascular imaging methods are improved by nanoparticles, which make heart tissues and blood arteries easier to see. The targeted accumulation of nanomaterials in particular cells and tissues, especially immune cells, has made them highly attractive for use in contrast agent administration in recent years [92]. There are multiple nanomaterial systems available for pre-clinical imaging, such as lipid-based nanoparticles [93], solid core polymeric nanoparticles [96] metallic nanoparticles [97], and amphiphilic polymers [98]. For MRI, iron oxide nanoparticles (IONPs) are a type of metallic nanoparticle that generate a molecular imaging signal. For PET, Technetium-99 (99Tc) is a type of small molecule that is encapsulated or conjugated in these nanoparticles. Ex vivo labelling [99] and the use of targeting moieties via antibodies or peptides [100] are two techniques that can be used to target single cells using nanoparticles. Iron oxide nanoparticles enhance magnetic resonance imaging's contrast, allowing for more precise imaging of myocardial infarction and vascular anomalies [21]. An updated overview of the area is necessary, nonetheless, given the quick advancement of pre-clinical research in this area of cardiovascular imaging and the recent advances in nanoparticle targeting to particular cell types.

Regenerative Medicine

Scaffolds supporting injured heart tissue regeneration are created with nanomaterials [28]. Following a heart attack, these scaffolds can release growth factors and other therapeutic agents, which encourage tissue repair and enhance cardiac function.

6.3 Neurodegenerative Disorders

Neurodegenerative illnesses like Parkinson's and Alzheimer's may be diagnosed and treated with the help of nanomedicine. Selenium, gold, copper oxides, copper and other nanoparticles are utilized in diagnosis and drug delivery systems for neurodegenerative diseases [29] (Fig. 2a).

Drug Delivery Across the Blood-Brain Barrier (BBB)

One of the major limitations in managing neurodegenerative diseases is delivering drugs across the blood-brain barrier. It is possible to engineer nanoparticles to get past this barrier and give therapeutic substances straight to the brain. For instance, neuroprotective medications can be better delivered to specific brain locations by encapsulating them in polymeric nanoparticles and liposomes [30].

Neuroimaging

By enhancing neuroimaging methods, nanoparticles enable the early detection and tracking of neurodegenerative illnesses. By improving the contrast in MRIs, magnetic nanoparticles can provide fine-grained pictures of anomalies and brain structures [31].

Gene Therapy

Genetic materials, like DNA or siRNA, can be delivered to particular neurons using nanoparticles. This may contribute to regulating the expression of genes implicated in neurodegenerative processes, providing a possible treatment strategy for illnesses such as ALS and Huntington's disease [30].

6.4 Infectious Diseases

Nanomedicine offers innovative approaches to prevent, diagnose, and treat infectious diseases (Fig. 2b).

Antimicrobial Nanoparticles

Strong antibacterial properties make silver nanoparticles useful for coating medical devices and in wound dressings to stop infections [32]. Due to the antimicrobial property of silver alone, it is well suited for the bio fabrication and enhance the antimicrobial properties of synthesized silver nanoparticle by encapsulating bioactive compounds. Additionally, they can be used in formulations to treat fungal, viral, and bacterial illnesses. In a recent study, it has been revealed that bio fabricated silver nanoparticle from aquous extract of *P. oxalicum* exerted significant antimicrobial properties against pathogenic bacterial strains such as *Escherichia coli* and *Streptococcus aureus* as well as pathogenic fungal strains such as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus luchuensis* and *Penicillium albicans* [41].

Vaccine Delivery

By improving the stability and delivery of the antigen, nanoparticles are used to create more effective vaccinations. Lipid nanoparticles, for instance, are employed in mRNA vaccines, like the ones created for COVID-19, to shield and transport the mRNA to immune cells [33, 34]. Another cutting-edge technique for the successful in vivo delivery of an exogenous mRNA vaccination is the use of lipid nanoparticles, or LNPs [102]. Nanoparticle based immunotherapy includes the use of synthetic antigen presenting cells (APCs) and nano vaccines. Dendritic cells, or APCs, are exposed to a variety of adjuvants and tumor-associated antigens through the use of nano vaccines [71].

Diagnostic Tools

The sensitivity and specificity of diagnostic tests for infectious diseases are enhanced by nanoparticles (Fig. 3a). Green-based nanoparticles are widely used in diagnosis, wound healing, and the identification of contaminates and other pathogens, among many other applications [71]. There are several benefits to using biological sources instead of current synthetic approaches for the creation of metal nanoparticles. Green NPs have been investigated for their possible use in therapeutic applications; these include antibacterial applications, drug delivery, medication administration, biocompatibility, and cancer diagnostics and treatment [102]. For example, lateral flow tests use gold nanoparticles to quickly detect viruses such as HIV and influenza [35].

6.5 Orthopaedics

Nanomedicine has applications in the treatment and management of orthopedic conditions, enhancing bone repair and joint health (Fig. 3b).

Bone Regeneration

Scaffolds made of nanomaterials are utilized to promote bone regeneration. The natural bone matrix is mimicked by hydroxyapatite nanoparticles and nanocomposites, which encourage osteoblast adhesion and differentiation—two processes essential to bone regeneration [36].

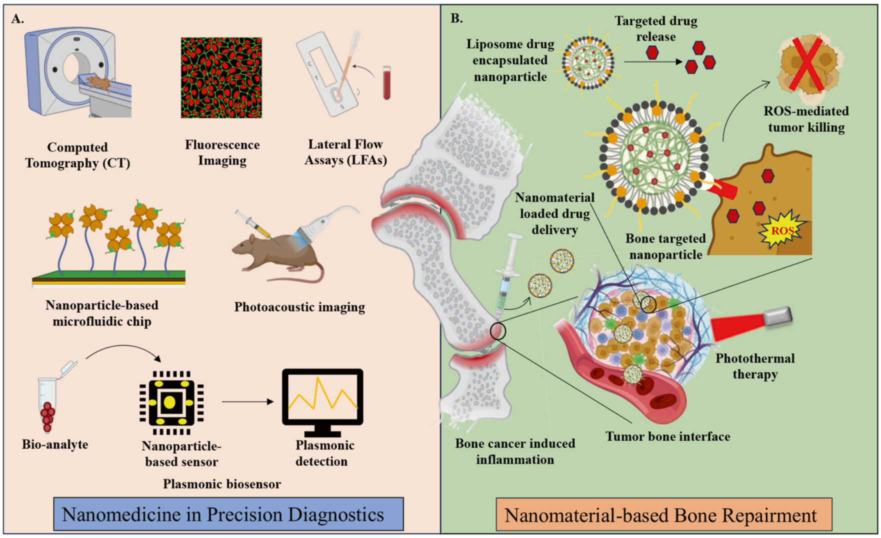
A.
Computed Tomography (CT)
Fluorescence Imaging
Lateral Flow Assays (LFAs)
Nanoparticle-based microfluidic chip
Photoacoustic imaging
Bio-analyte
Nanoparticle-based sensor
Plasmonic detection
Plasmonic biosensor
Nanomedicine in Precision Diagnostics
B.
Liposome drug encapsulated nanoparticle
Targeted drug release
ROS-mediated tumor killing
Nanomaterial loaded drug delivery
Bone targeted nanoparticle
ROS
Photothermal therapy
Bone cancer induced inflammation
Tumor bone interface
Nanomaterial-based Bone Repairment

Fig. 3 Diagrammatic illustration of nanomedicine-based theranostic approach. (**a**) Nanomedicine in precision diagnostics: Various imaging and biosensor techniques that utilize the nano-scale system has been applied for specific targeting and detection of pre diagnosis of bone diseases. (**b**) Nanomaterial-based bone repairment: The liposomal encapsulation of drug is done for the multifunctional targeting and controlled drug release. The photothermal therapy has been aimed to mitigate the bone cancer induced inflammation via ROS mediated active killing of tumor cells

Drug Delivery

Drugs can be delivered to bone tissues directly by nanoparticles, improving the efficacy of treatments for diseases like osteoporosis and bone infections. To target bone infections and encourage recovery, calcium phosphate nanoparticles, for example, can be loaded with antibiotics or bone-growth hormones [37].

Joint Repair

Advanced implants and prosthetics are developed using nanomaterials. Joint implant nano coatings can extend life and improve the functionality of the devices by lowering wear and tear, improving biocompatibility, and preventing infections [38].

The treatment landscape is changing in many ways due to the advent of nanomedicine. It addresses some of the most difficult medical problems and greatly improves patient outcomes by leveraging the unique properties of nanomaterials to create targeted, efficient, and minimally invasive treatment solutions. The uses of nanomedicine are anticipated to grow with the development of science and technology, providing cutting-edge and potent therapies down the road.

7 Challenges and Future Directions

While nanomedicine holds immense promise, some challenges need to be addressed, such as:

7.1 Regulatory and Safety Concerns

The main issue with this field is the development of a proper regulatory framework for the assessment of nanomedicines. It is highly crucial to guarantee that these all-new technologies provide total safety and reliability. Comprehensive and penetrating evaluation and risk assessment should be created to make sure that the toxicology effects and environmental consequences of nanomaterials have been duly weighed and recognized. Agencies' collaboration with researchers and industry stakeholders is the primary factor to establish the same standard and constitute guidelines for products that are based on nanomedicine.

7.2 Ethical and Social Implications

Because of the exciting developments in the field of nanomedicine ethical clearance and social problems appear, which shouldn't be underestimated. For example, the type of nanotechnology source therapies needs to be provide the same access for everyone, informed consent during the care process when it comes to nanomedicine must be carefully considered and the privacy of personal data which stems from genetic information needs to also be taken into account. The possibility of unexpected outcomes, which could be something like the of human capacities above the therapeutic goals, again raises ethical questions and requires open discourse with society.

Although multiple obstacles must be overcome, nanomedicine is a promising discipline for pushing the boundaries of therapeutic technologies. The marriage of nanotechnology with the forthcoming fields such as artificial intelligence, machine information, and bioelectronics design the possibility of establishing sophisticated nanocomposites and custom medicine technologies. For example, combining nanomedicine with gene editing techniques including Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) may make feasible many custom gene treatments as well as regenerative medicine solutions [39]. Furthermore, the progress of the industrial techniques in nanofabrication, including advanced 3D printing and self-assembly techniques, may endow the production of more complex required nanomaterials and nanodevices for medical applications on a big scale [40].

8 Conclusion

Nanomedicine is one of the most sought-after fields which is also leading the upcoming generation in diagnostics, prevention, and treatment with newly evolved techniques and approaches. Through using the characteristic features of materials at the nano-level nanomedicine has arisen with the chance to create completely fresh ideas for the mistreatment of many diseases. This chapter focussed on some fundamental concepts of nanoscience, how to make and functionalize the nanoparticles and their diagnostic and therapeutic applications; we also explored the extraordinary capabilities of nanoparticles in diagnostics specifically in enhanced imaging techniques, biosensors, and nano sensors that help detect diseases at an early and apt stage. Furthermore, we have described the application of nanomaterials in targeted drug delivery and regenerative medicine, and their role in cancer treatment, thus highlighting their role in increasing the effectiveness of medication given simultaneously with minimizing the side effects.

One of nanomedicine's most advantageous characteristics also includes its interdisciplinary nature, which is made possible by blending principles from materials science, biology, chemistry, and engineering. This multi-disciplinary sort of approach has laid the foundation for innovative inventions such as nano-theranostics devices and smart nanomaterials. The diagnostic, and therapeutic capabilities happen to be integrated within the theranostics nanoparticle devices as well as the smart nanomaterials are specially designed to have biologically specific responses or external input. Nanomedicine has a set of great prospects that are still facing many problems that need to be solved. The technologies present numerous regulatory, logistical, ethical, and societal concerns that should be addressed as they progress. Remember that ongoing research is aimed at enhancing biocompatibility, reducing toxicity, and increasing the accuracy of targeted delivery and controlled release of nanomedicine.

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